

Figure 3: Stabilized Zinzyme Ribozyme Motif

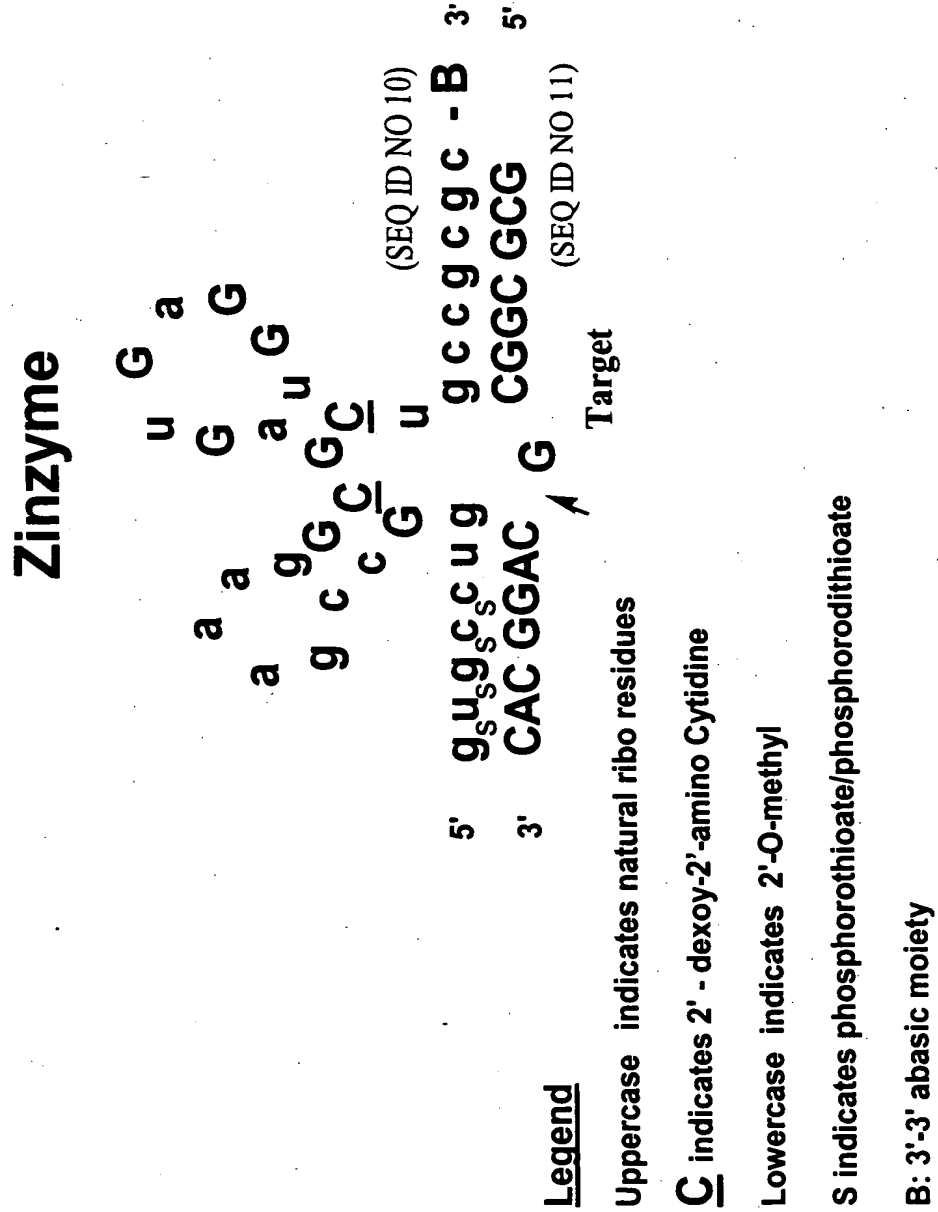
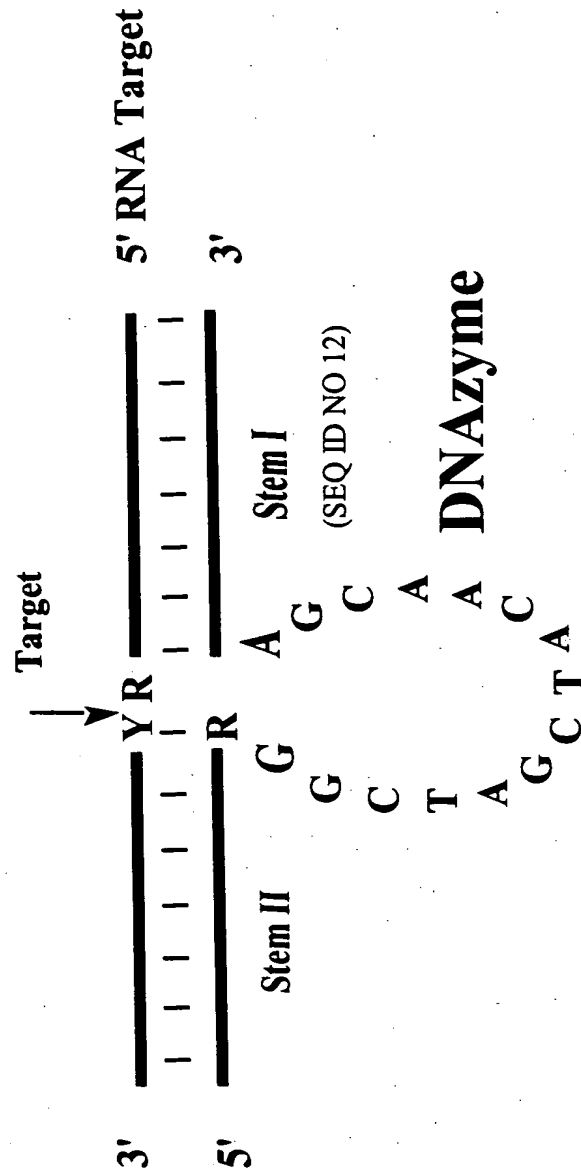


Figure 4: DNAzyme Motif



Legend

Y = U or C

R = A or G

Figure 5: Synthesis of Folate Linked phosphoramidite

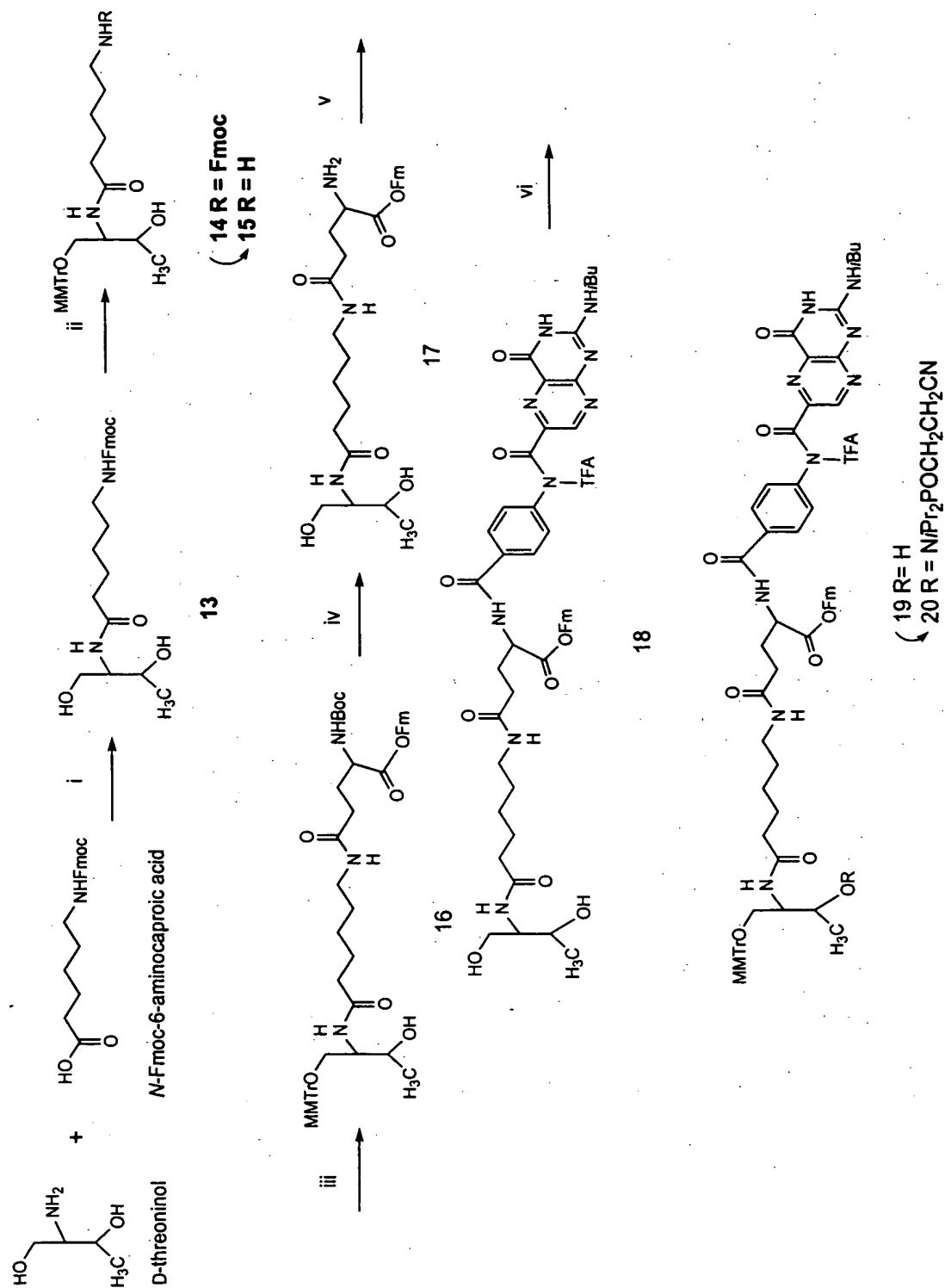


Figure 6: Fludarabine-Folate conjugates

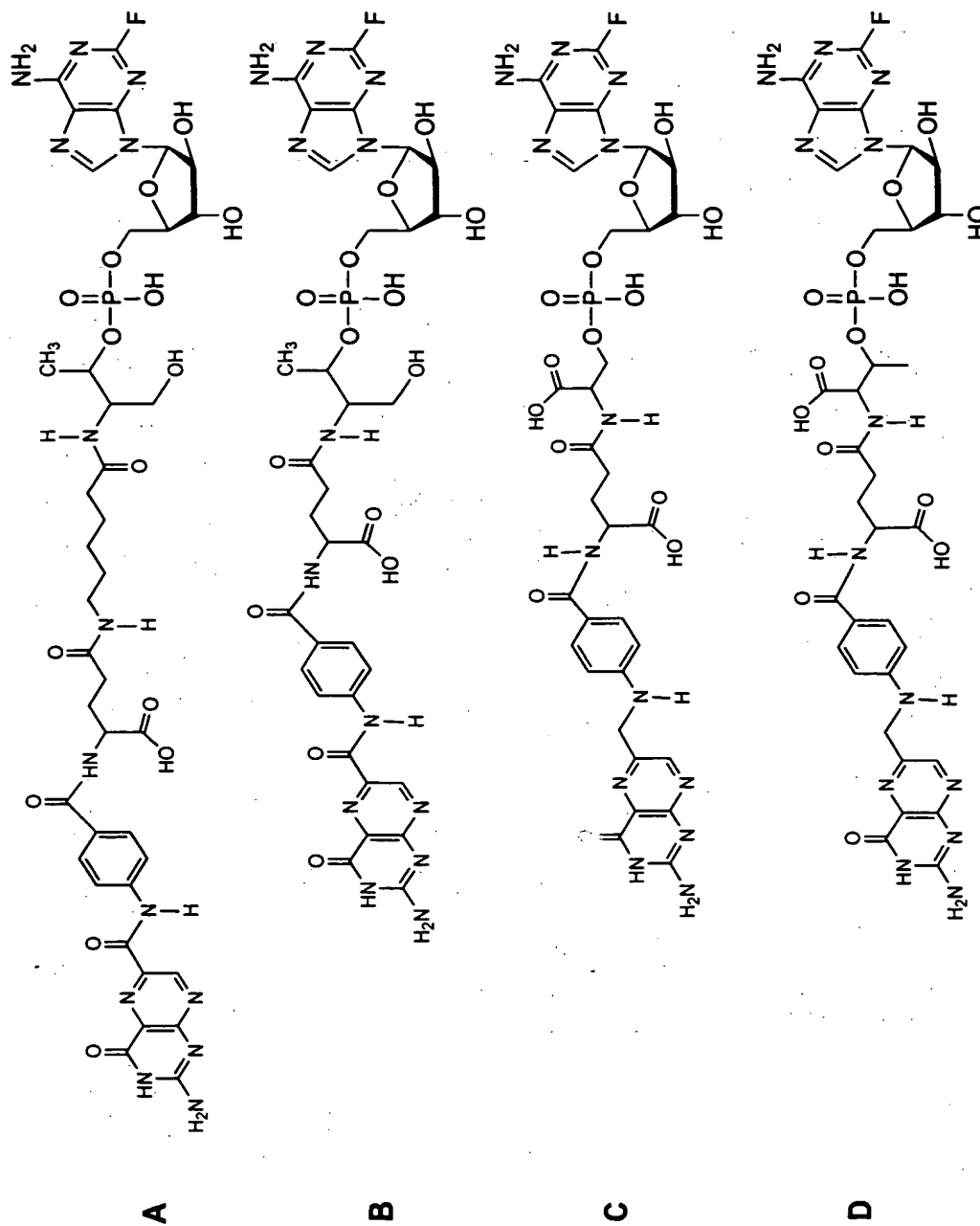


Figure 7: Solid Phase Post-synthetic conjugation of pterotic acid

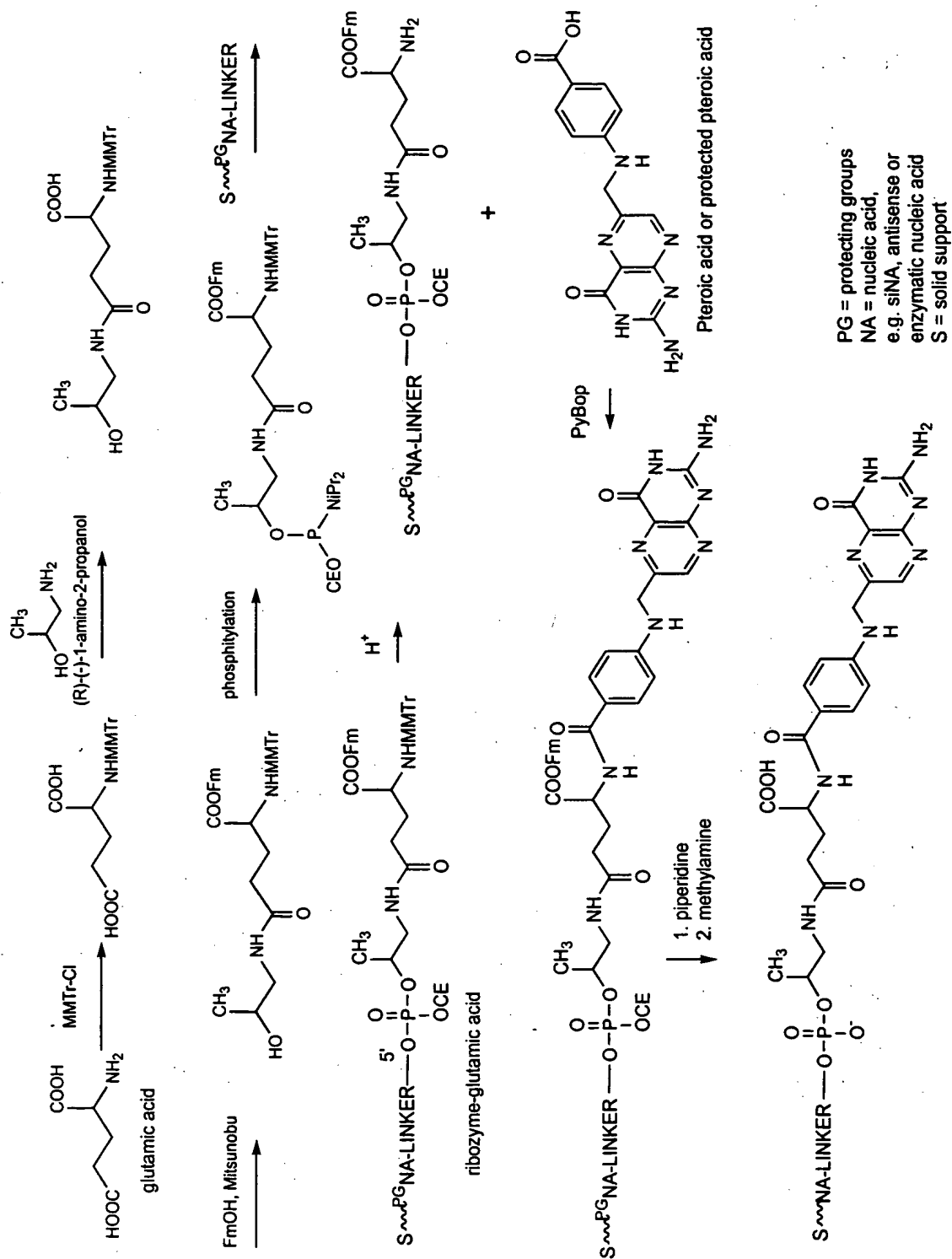


Figure 8: Chemo-enzymatic synthesis of pteronic acid synthon

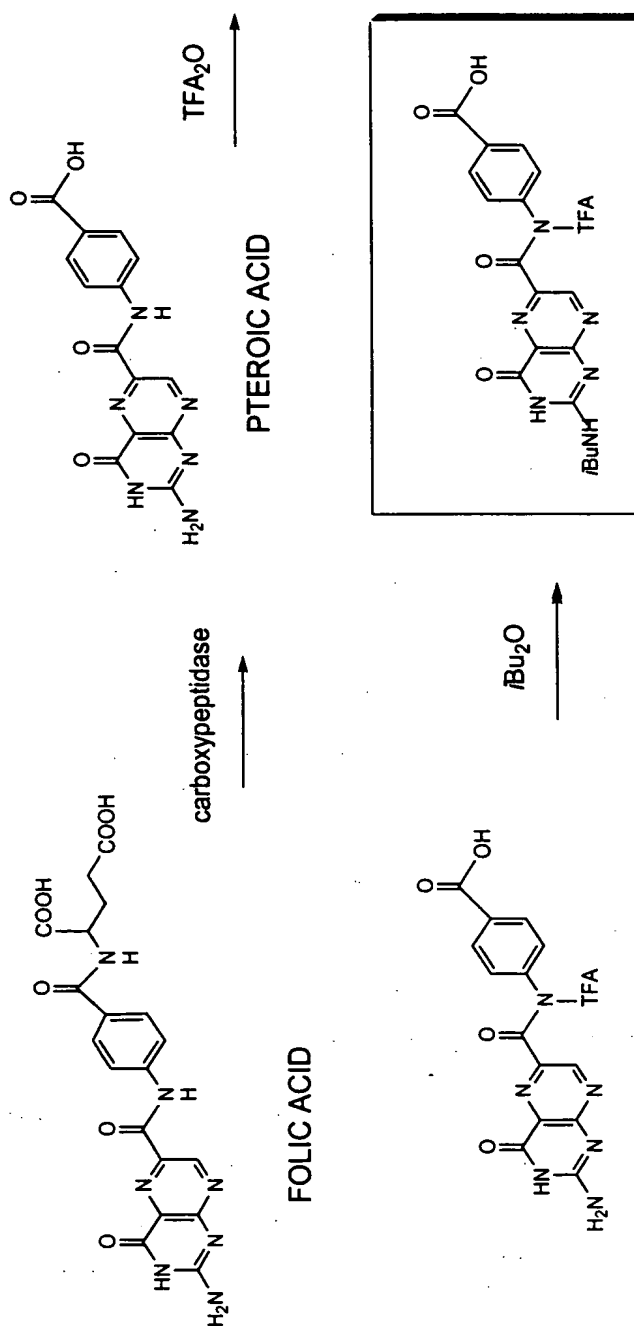
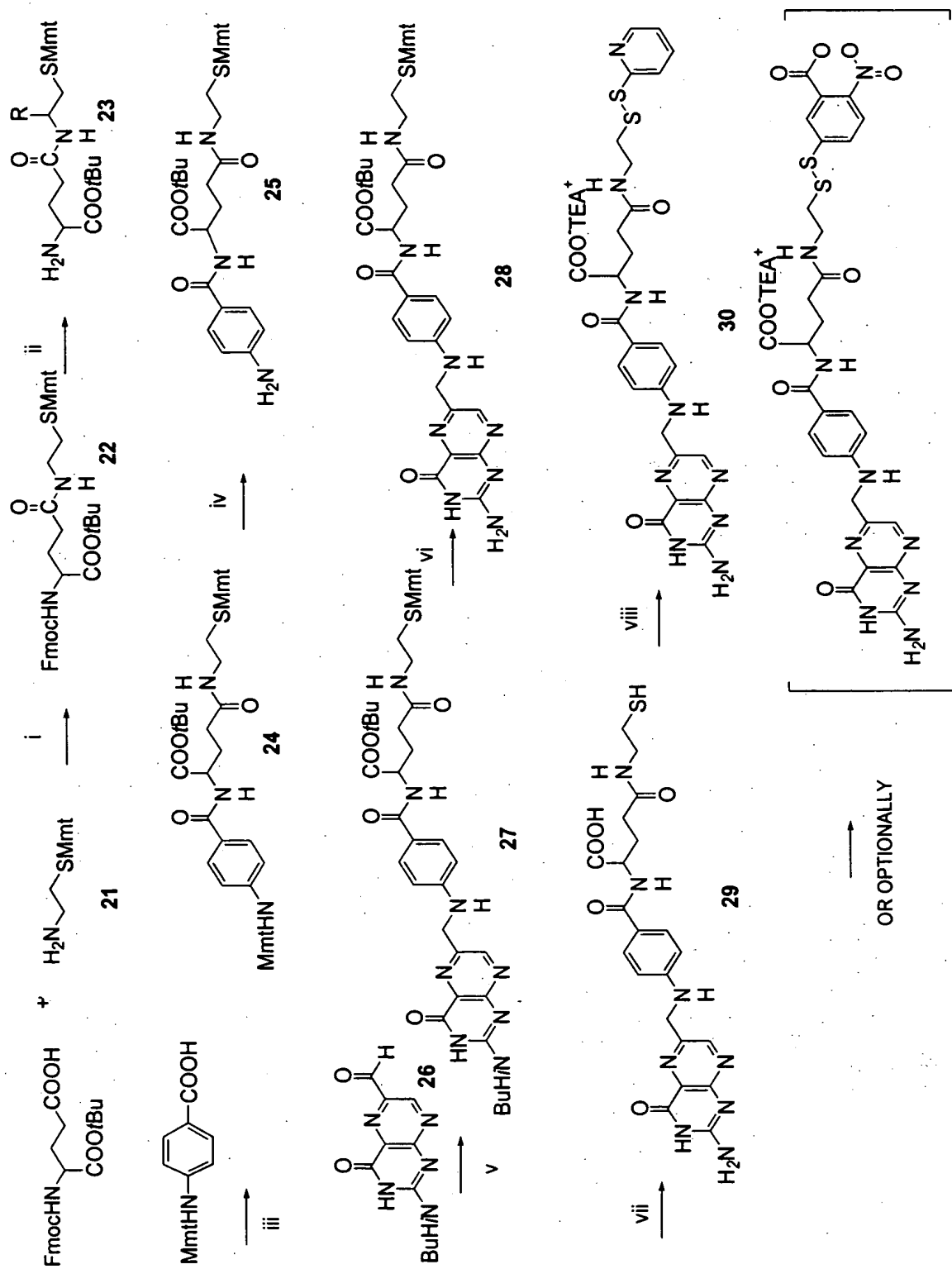


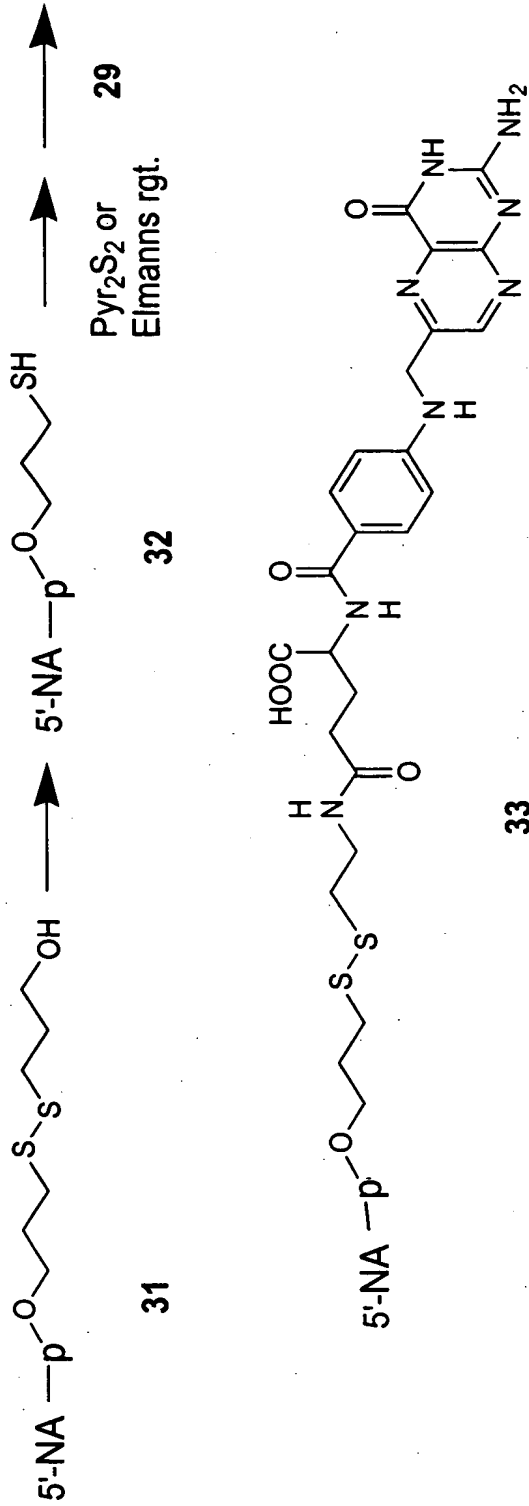
Figure 9



OR OPTIONALLY

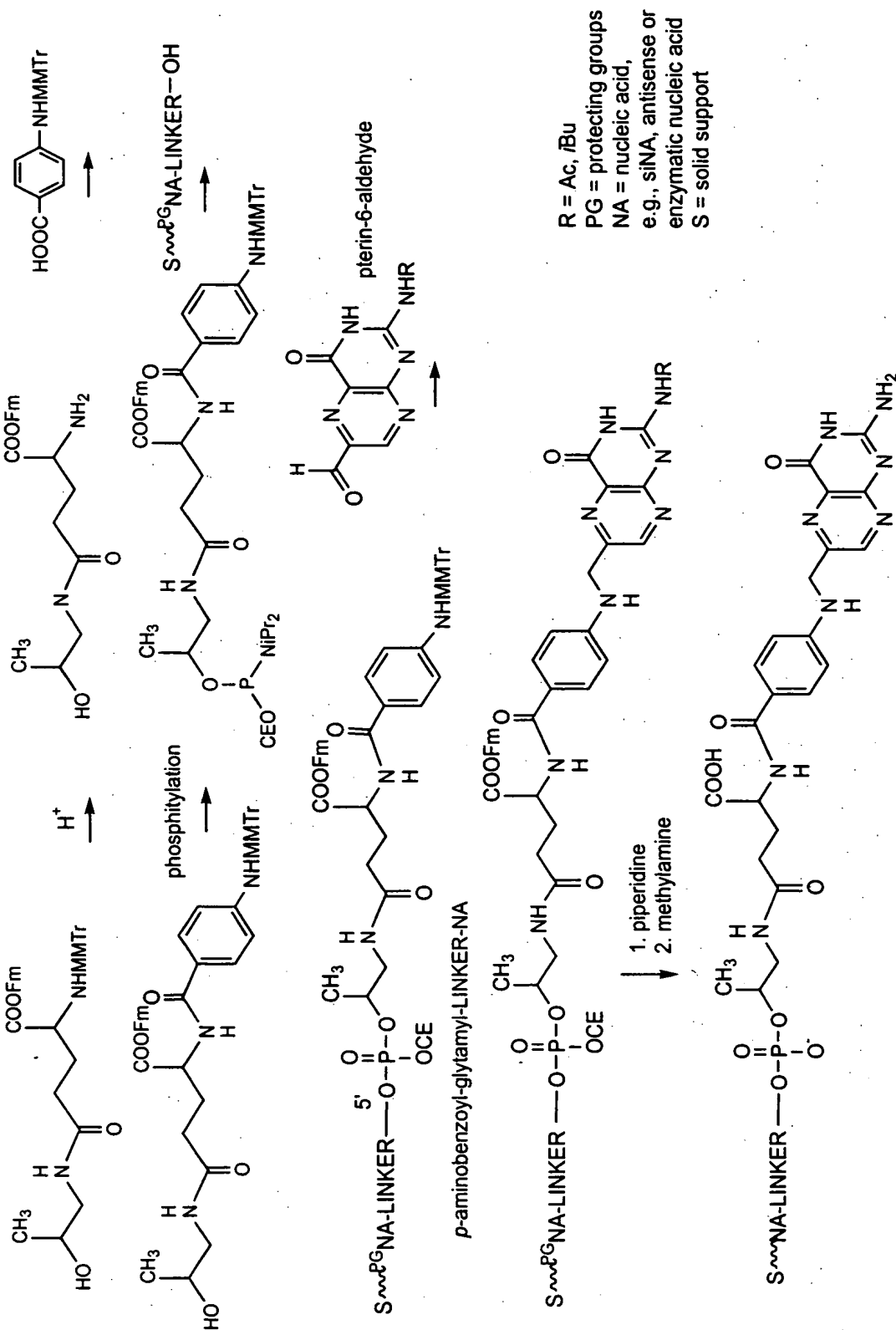


Figure 11



NA = Nucleic Acid, such as siNA, antisense, or enzymatic nucleic acid
 p = phosphorous moiety

Figure 12: Solid Phase Post-synthetic conjugation of pterioic acid



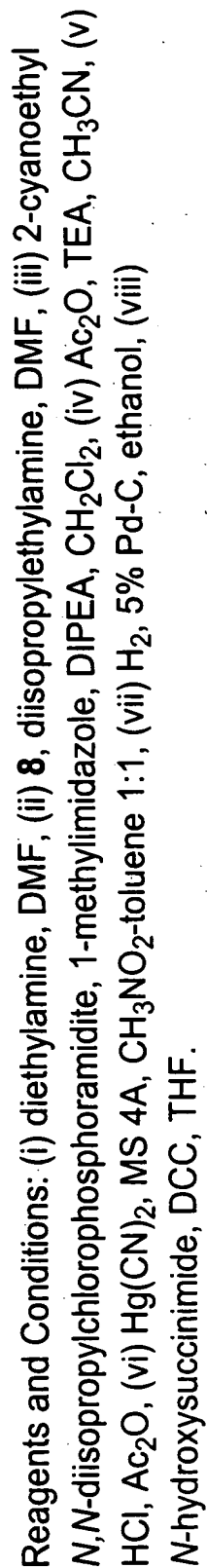
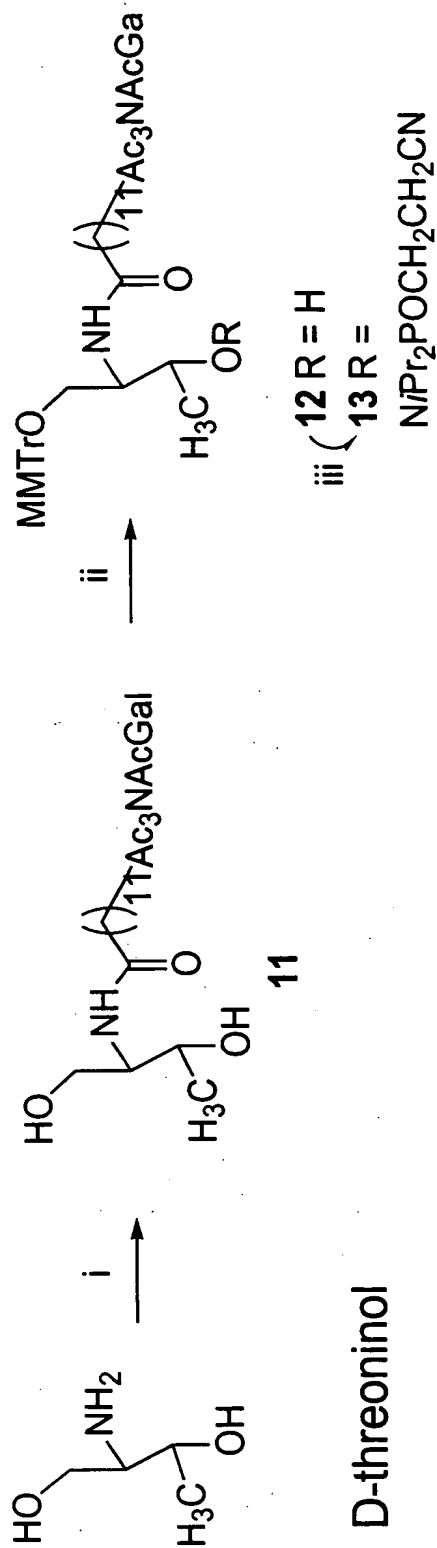
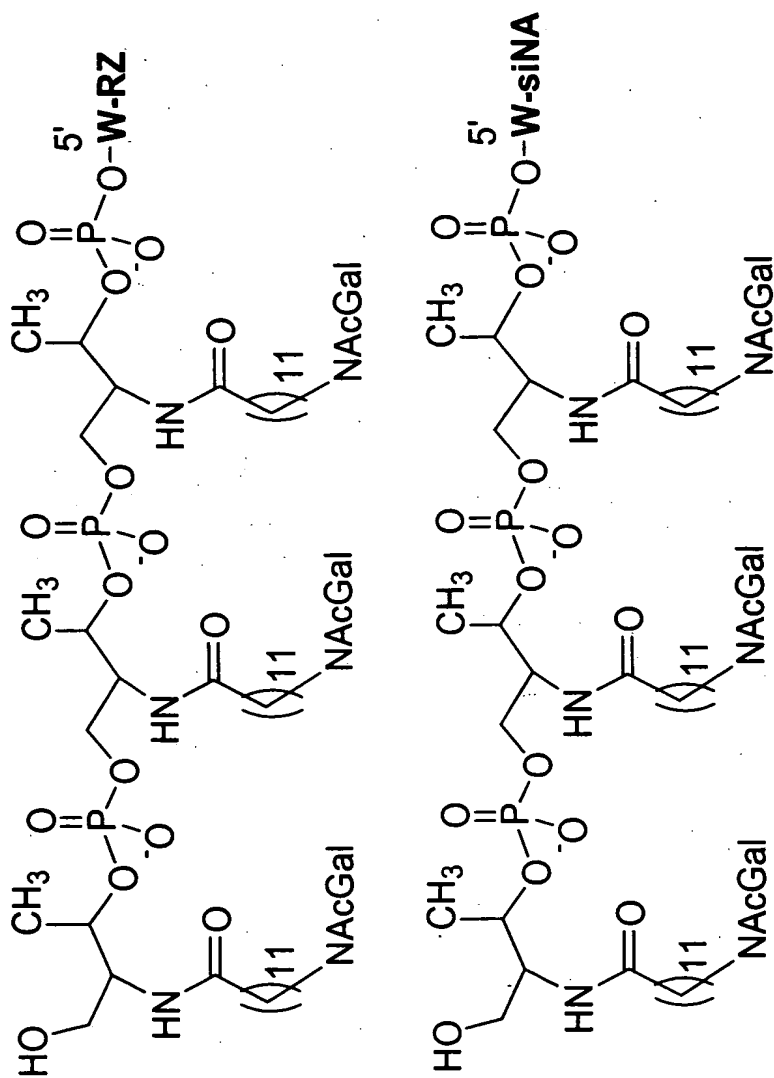


Figure 14: Synthesis of *N*-acetyl-*D*-galactosamine-*D*-threoninol conjugate



Reagents and Conditions: (i) 7, DCC, *N*-hydroxysuccinimide, (ii) MMTr-Cl, pyridine, (iii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, 1-methylimidazole, DIPEA, CH₂Cl₂.

Figure 15: Conjugation of targeting ligands to the 5'-end of a Ribozyme or siNA molecule



N-acetyl-D-galactosamine conjugate

Figure 16: Synthesis of dodecanoic acid linker

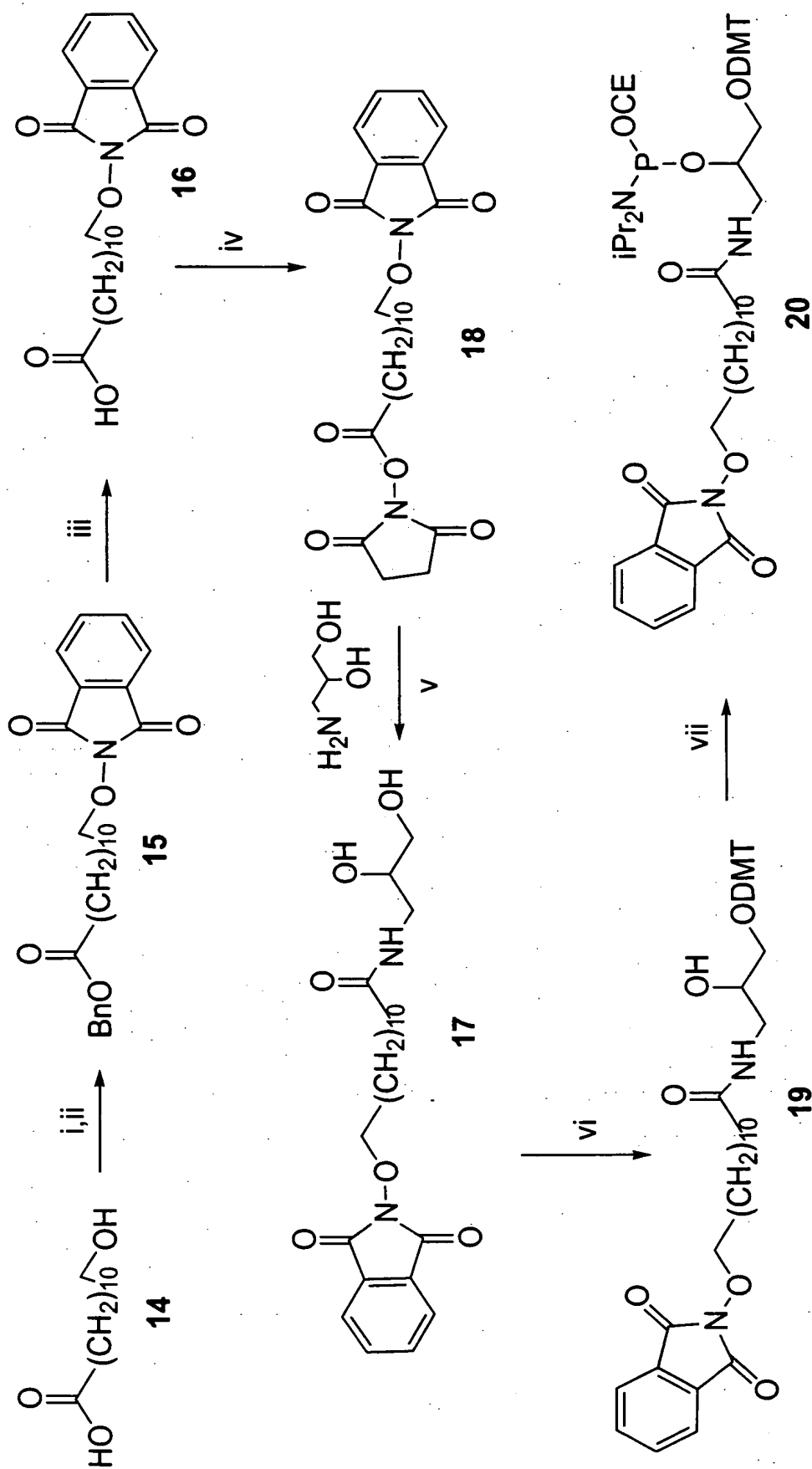


Figure 17: Oxime linked Nucleic Acid/Peptide Conjugate

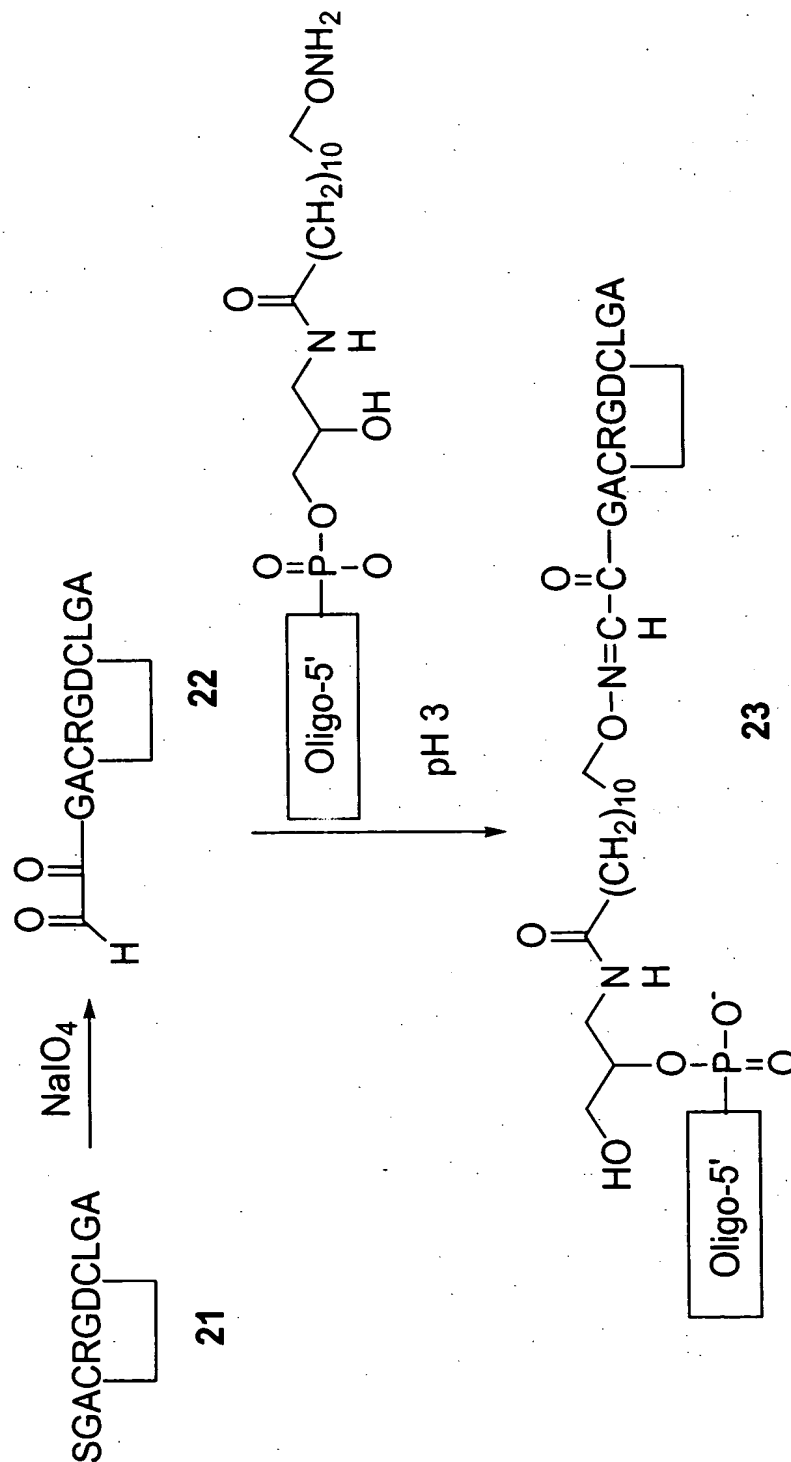
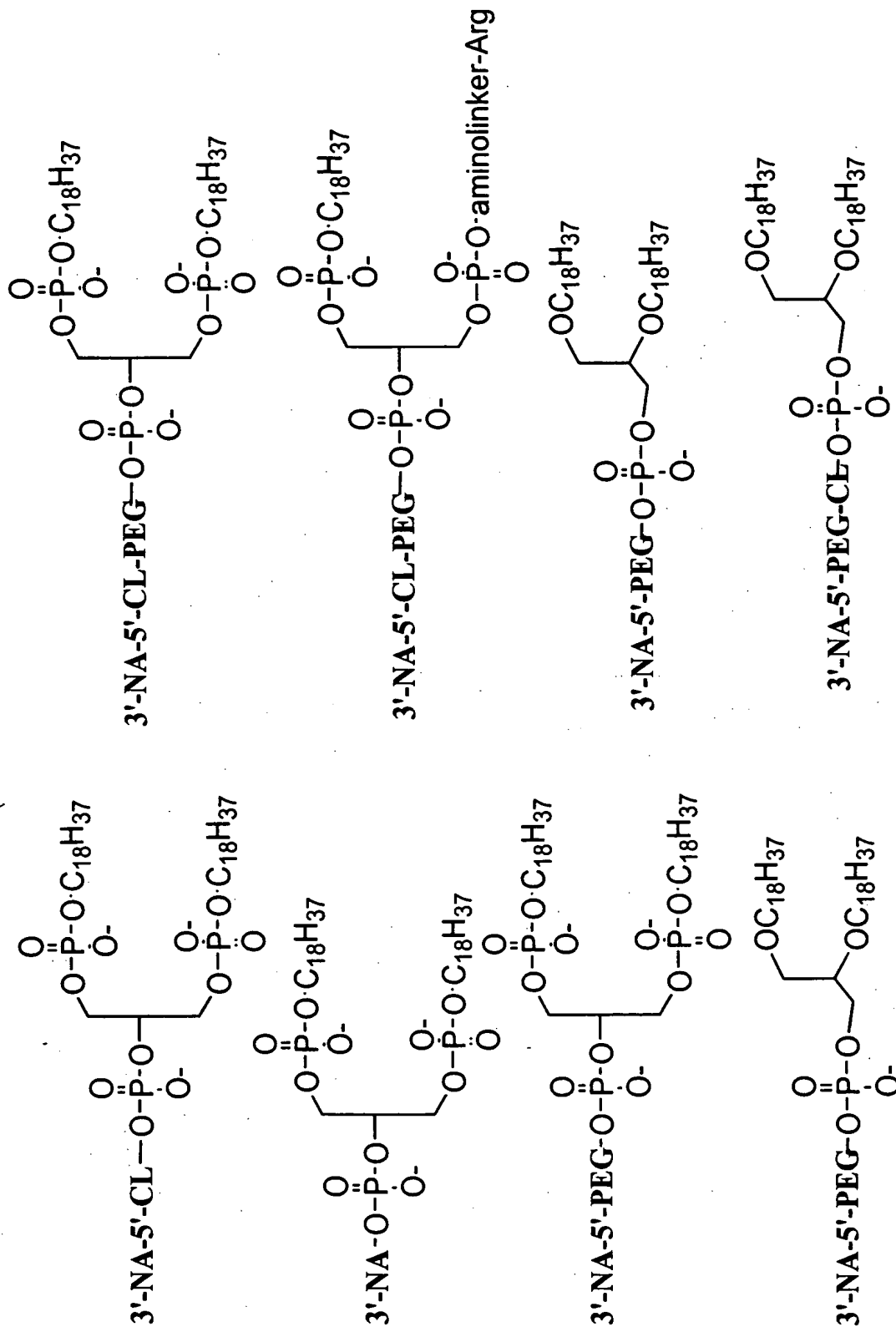


Figure 18: Nucleic Acid/Phospholipid Conjugates

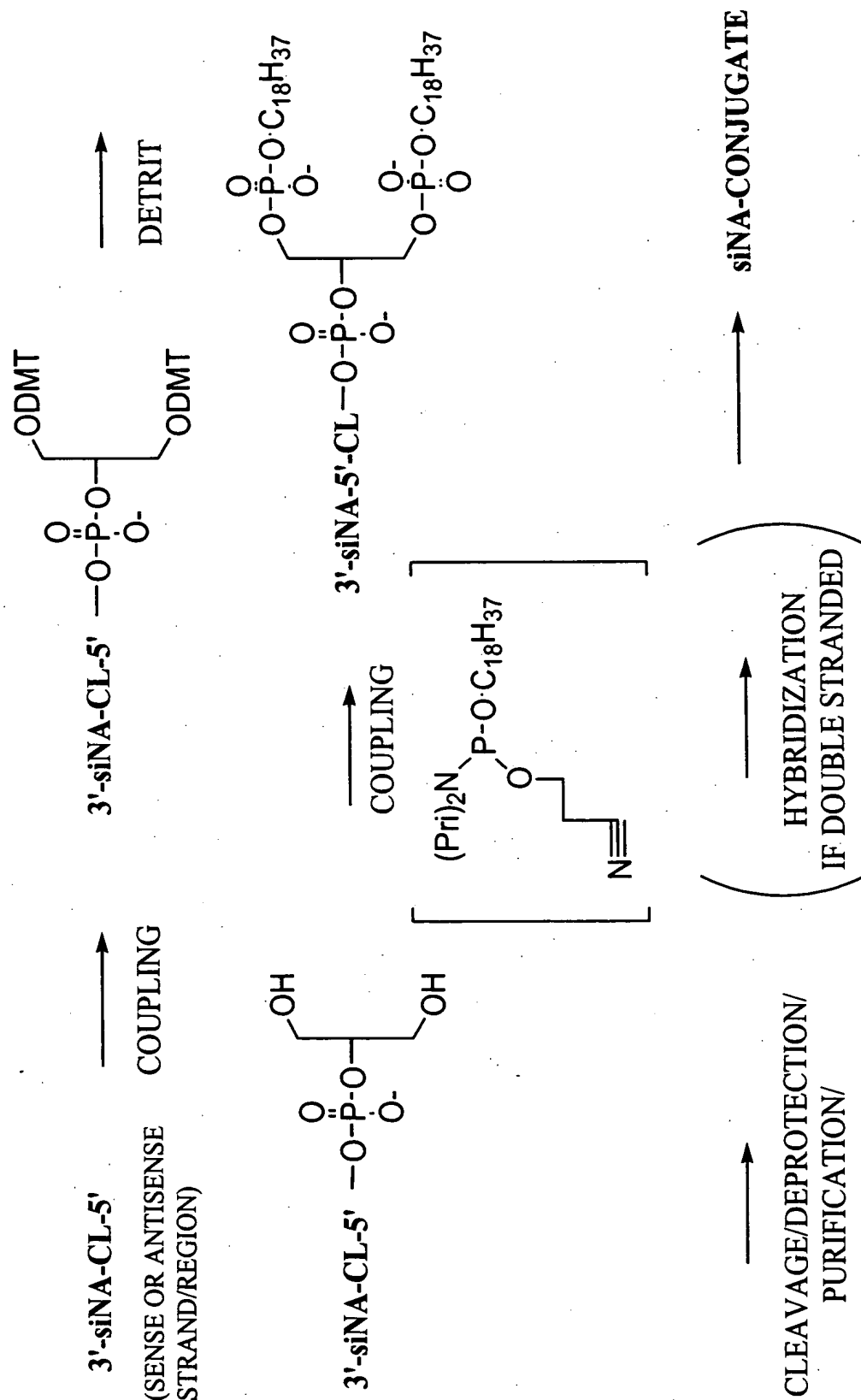


PEG=polyethylene glycol

CL=cleavable linker (e.g. A-dT, C-dT)

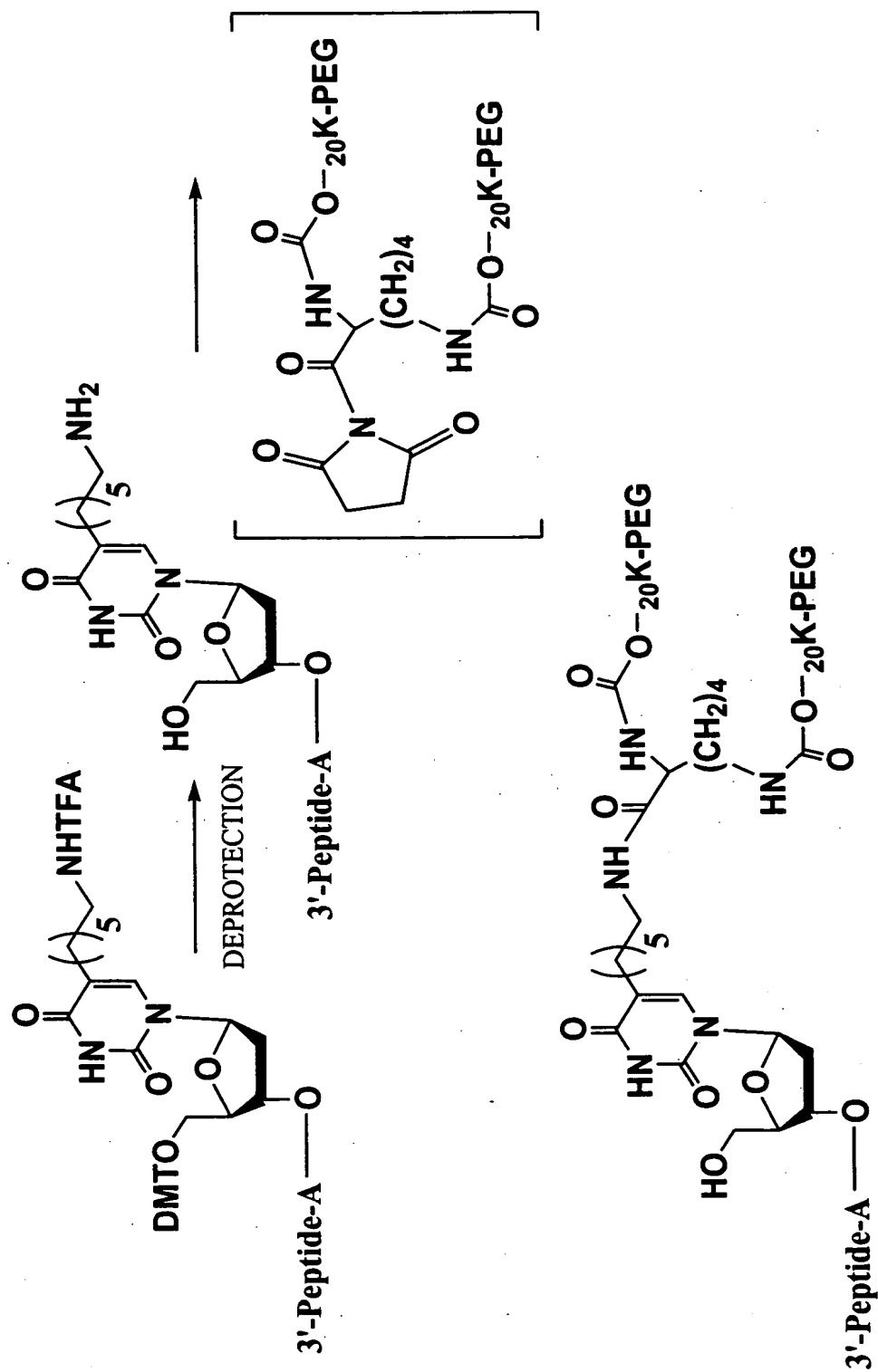
NA= Nucleic Acid Molecule such as siNA, antisense, or enzymatic nucleic acid

Figure 19: siNA Phospholipid Conjugate



CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMINE DIMER THAT IS OPTIONALLY PRESENT

Figure 20: Peptide PEG Conjugate



A = Adenosine

Figure 21: 40-KDa PEG-Angiozyme vs Angiozyme

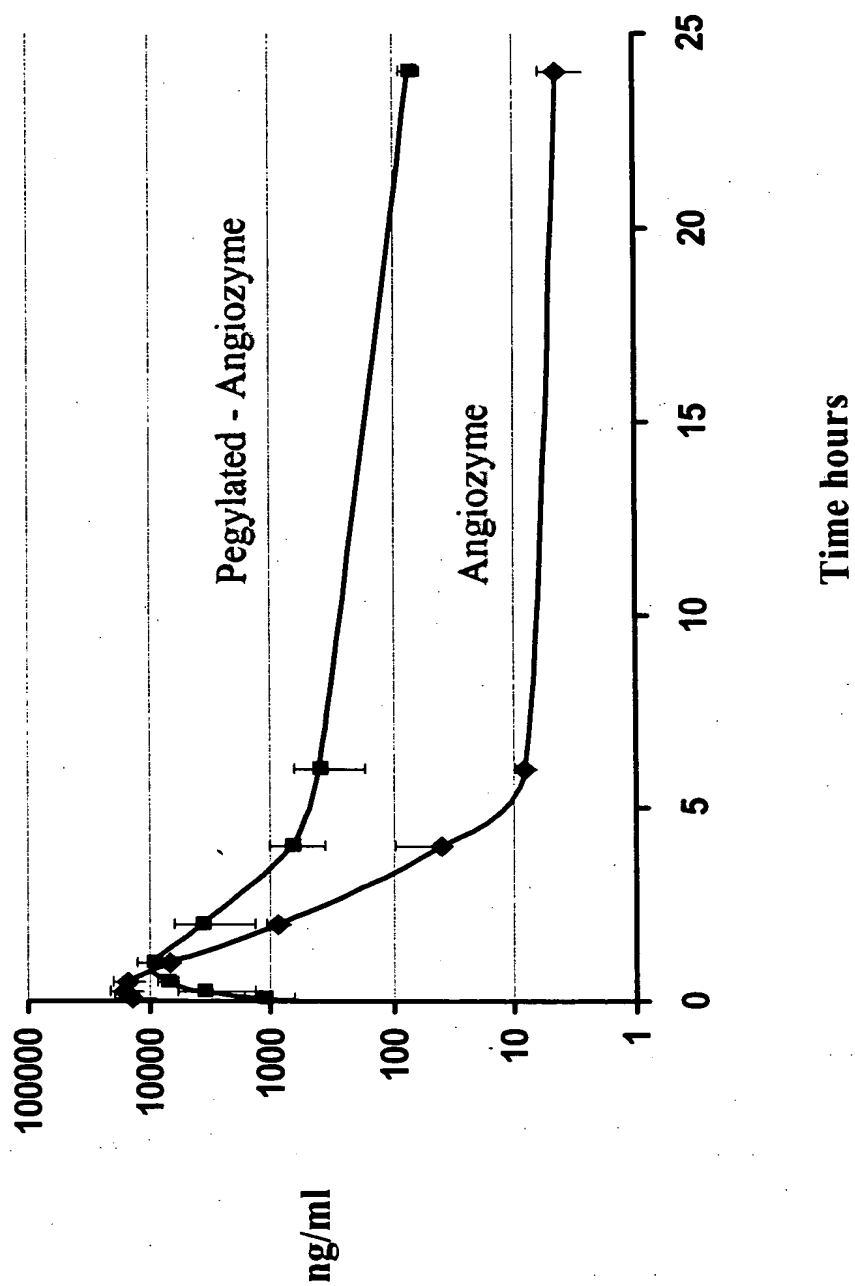


Figure 22: Phospholipid-Angiozyme vs Angiozyme

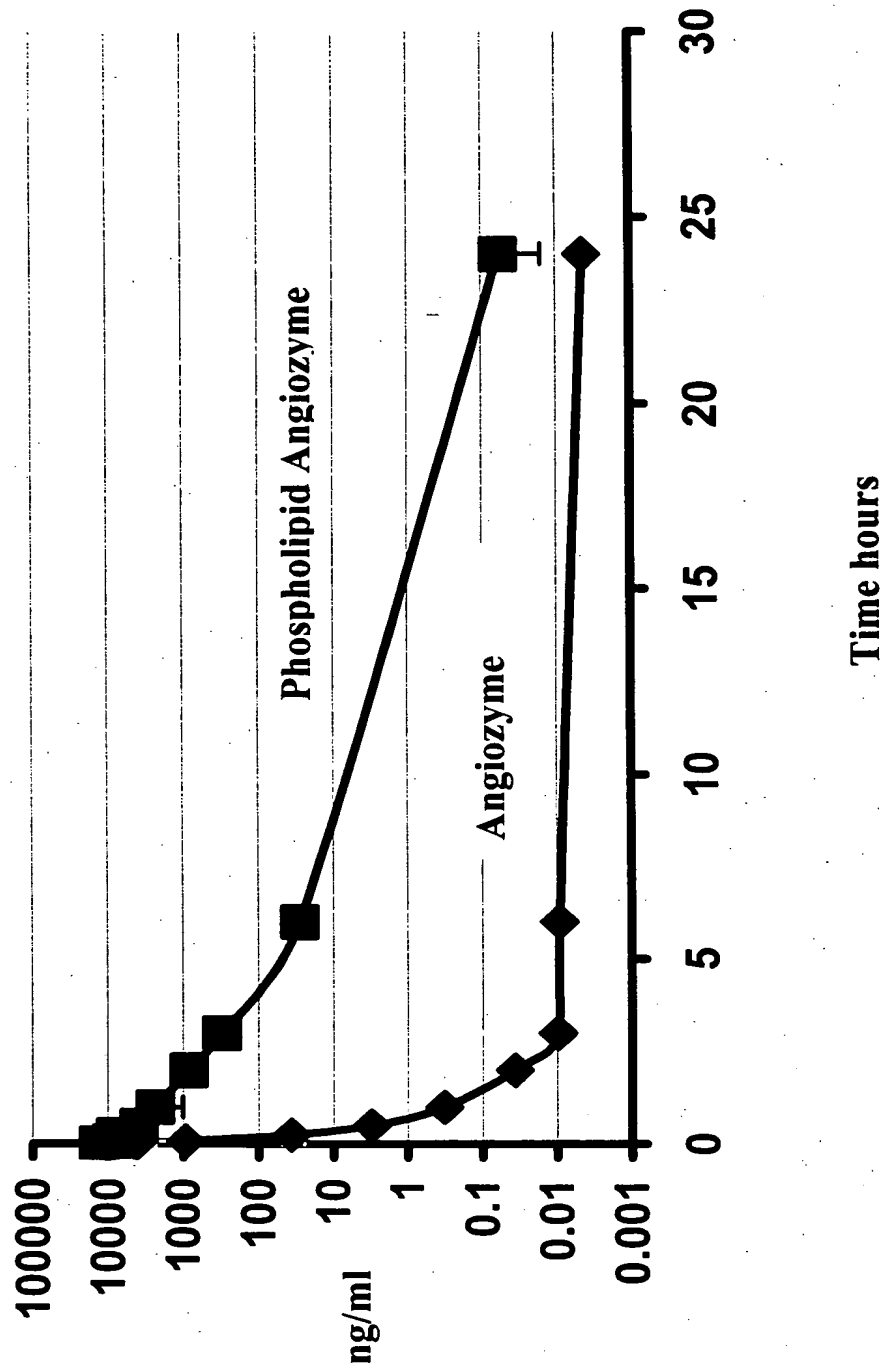
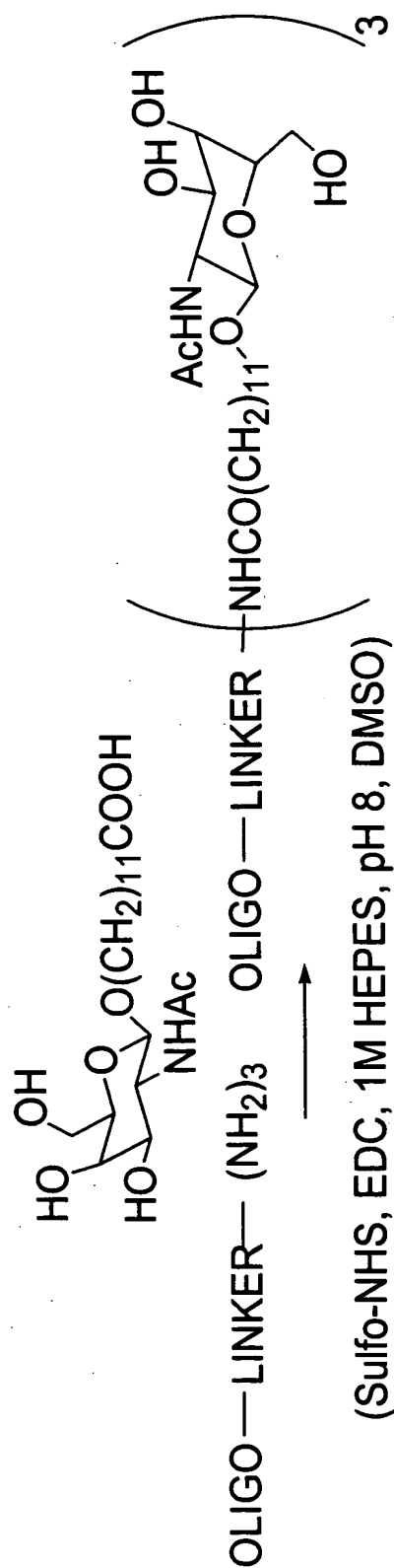
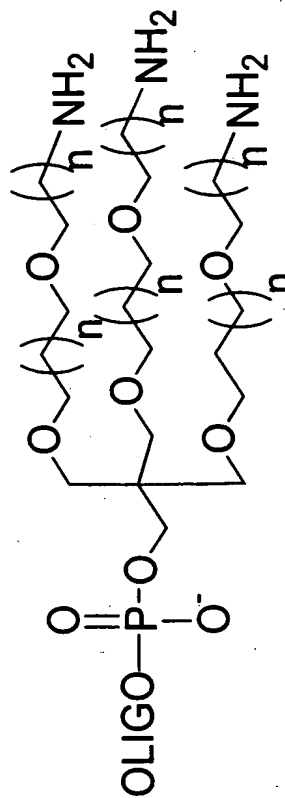


Figure 23: Oligonucleotide-NAcGalactosamine post-synthetic coupling



FOR EXAMPLE: OLIGO-LINKER =



Where n is an integer from 1 to 20

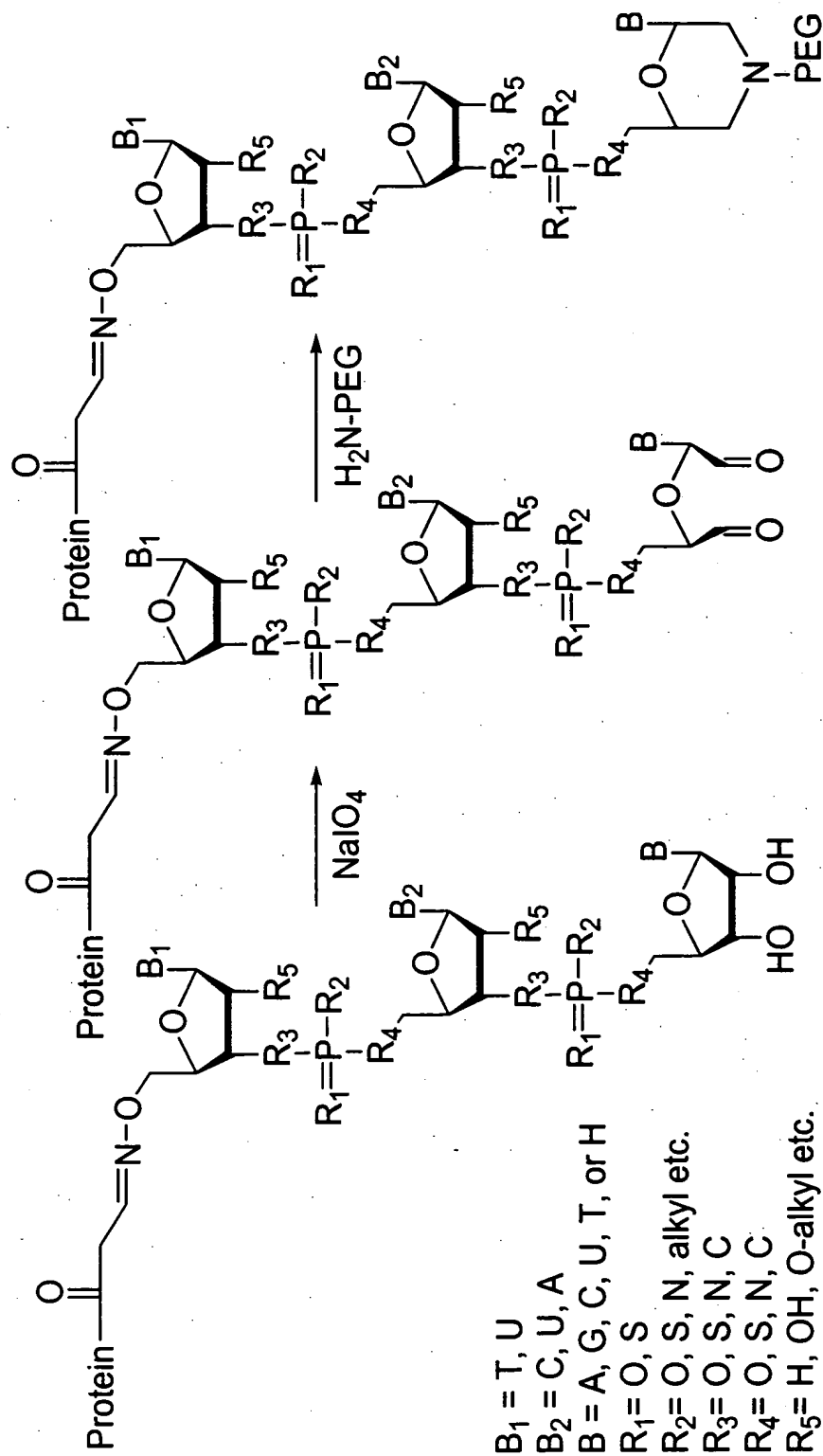


Figure 25: Protein PEG conjugate with cleavable linker

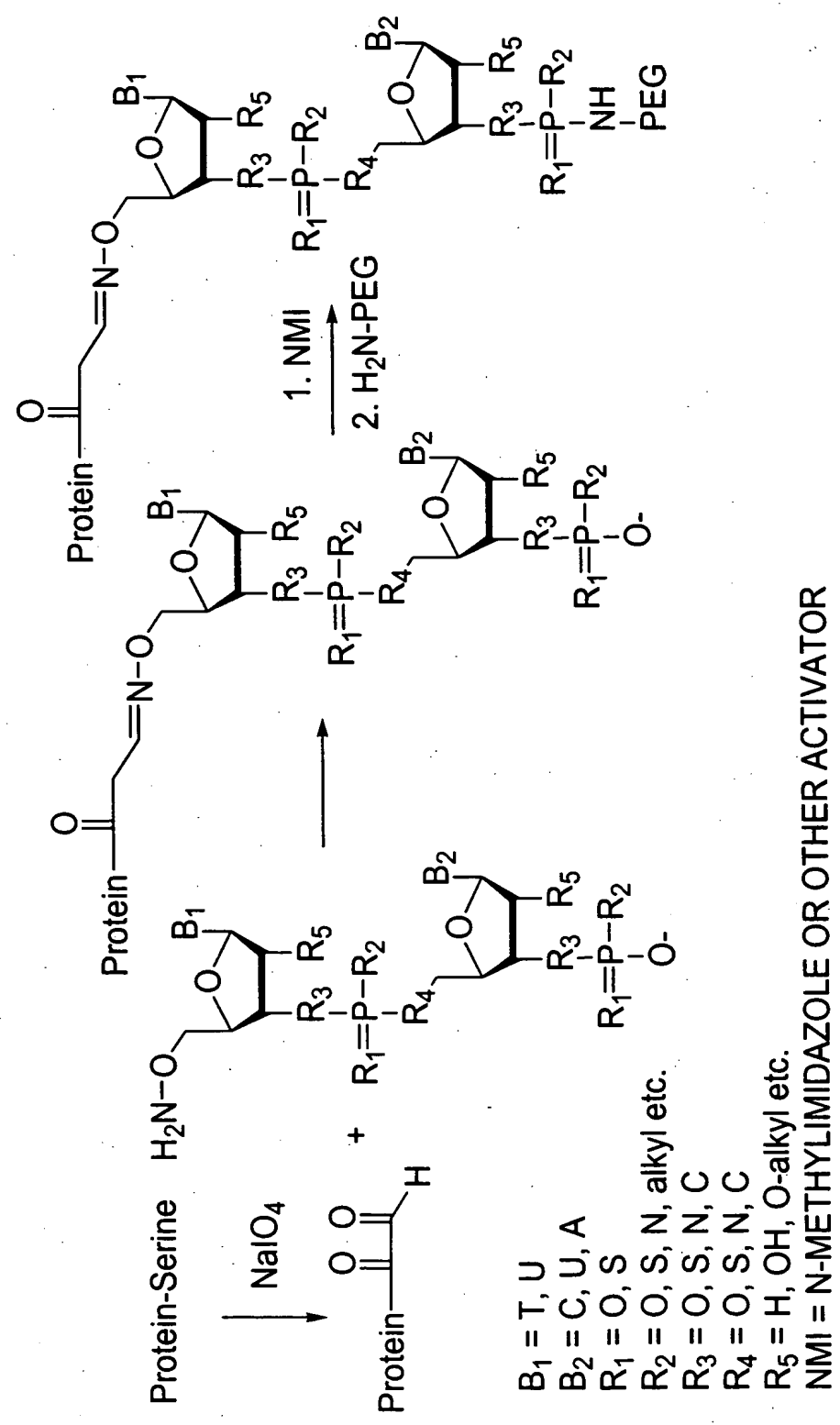
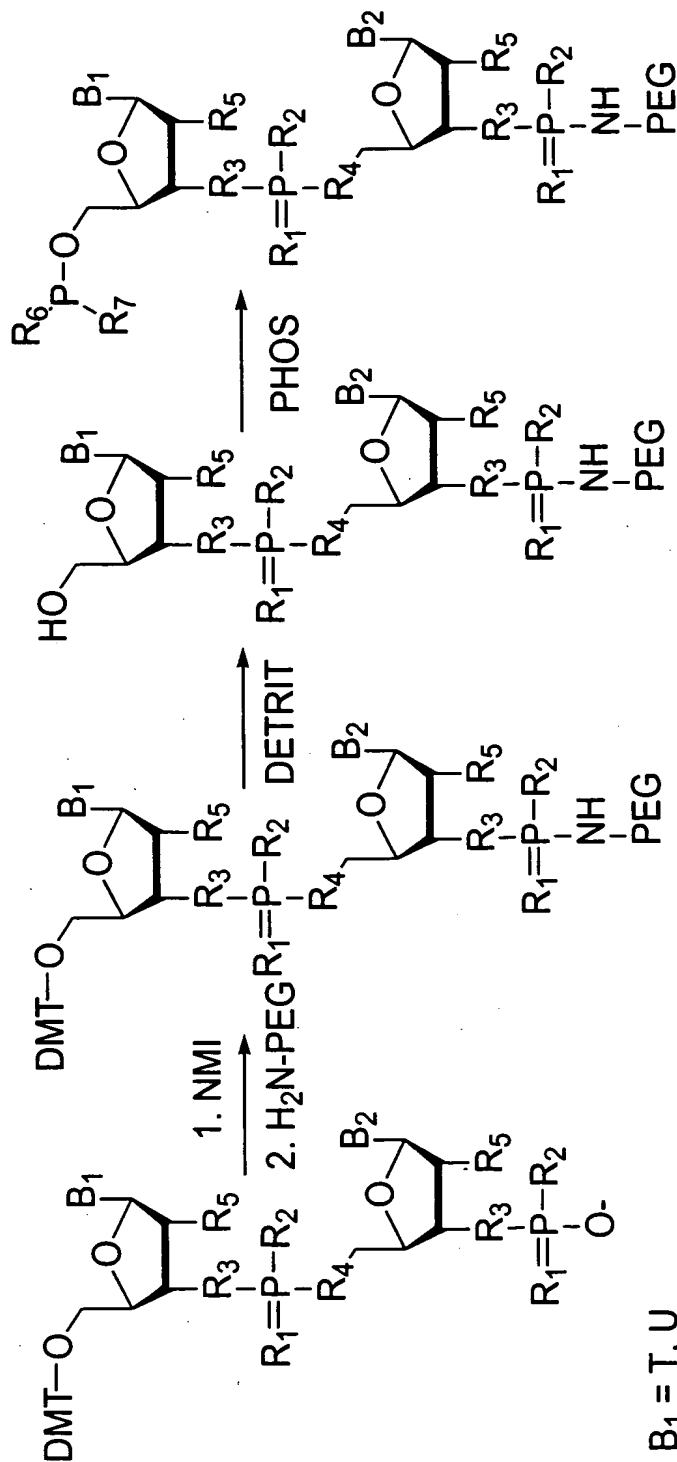


Figure 26a: PEG with cleavable linker



B₁ = T, U

B₂ = C, U, A

R₁ = O, S

R₂ = O, S, N, alkyl etc.

R₃ = O, S, N, C

R₄ = O, S, N, C

R₅ = H, OH, O-alkyl etc.

R₆ = substituted nitrogen, eg. N(iPr)₂

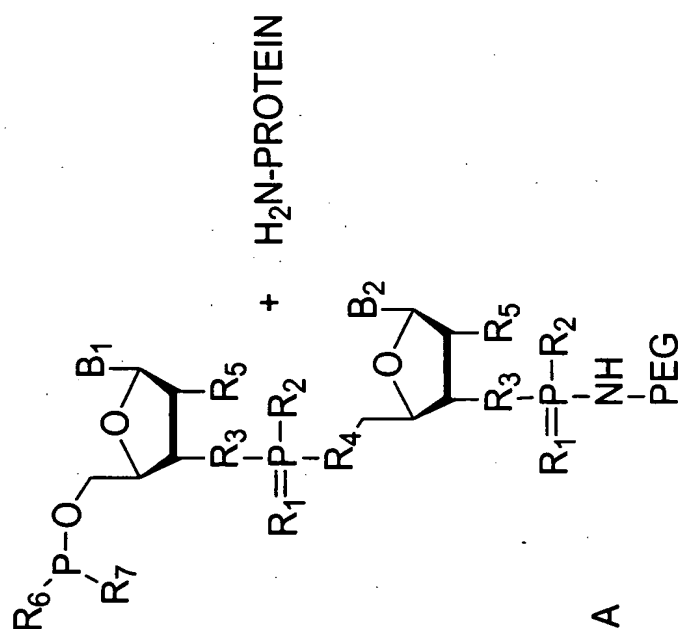
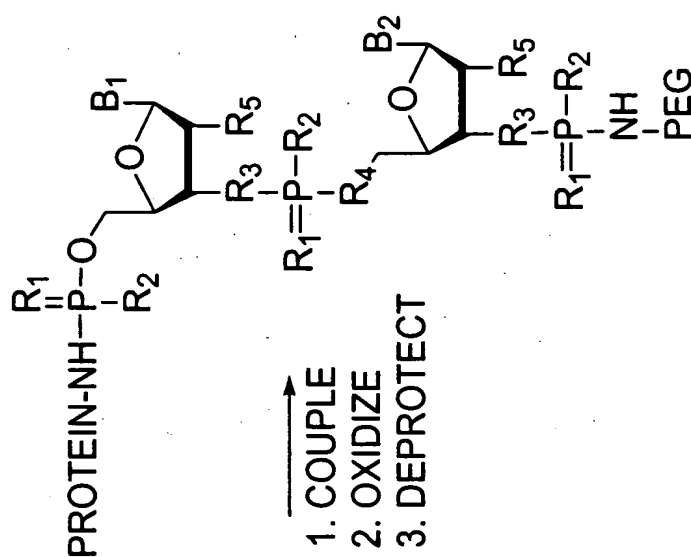
R₇ = O or S alkyl, cyanoalkyl, eg. cyanoethyl

PHOS = PHOSPHITYLATION

DET = DETRITYLATION

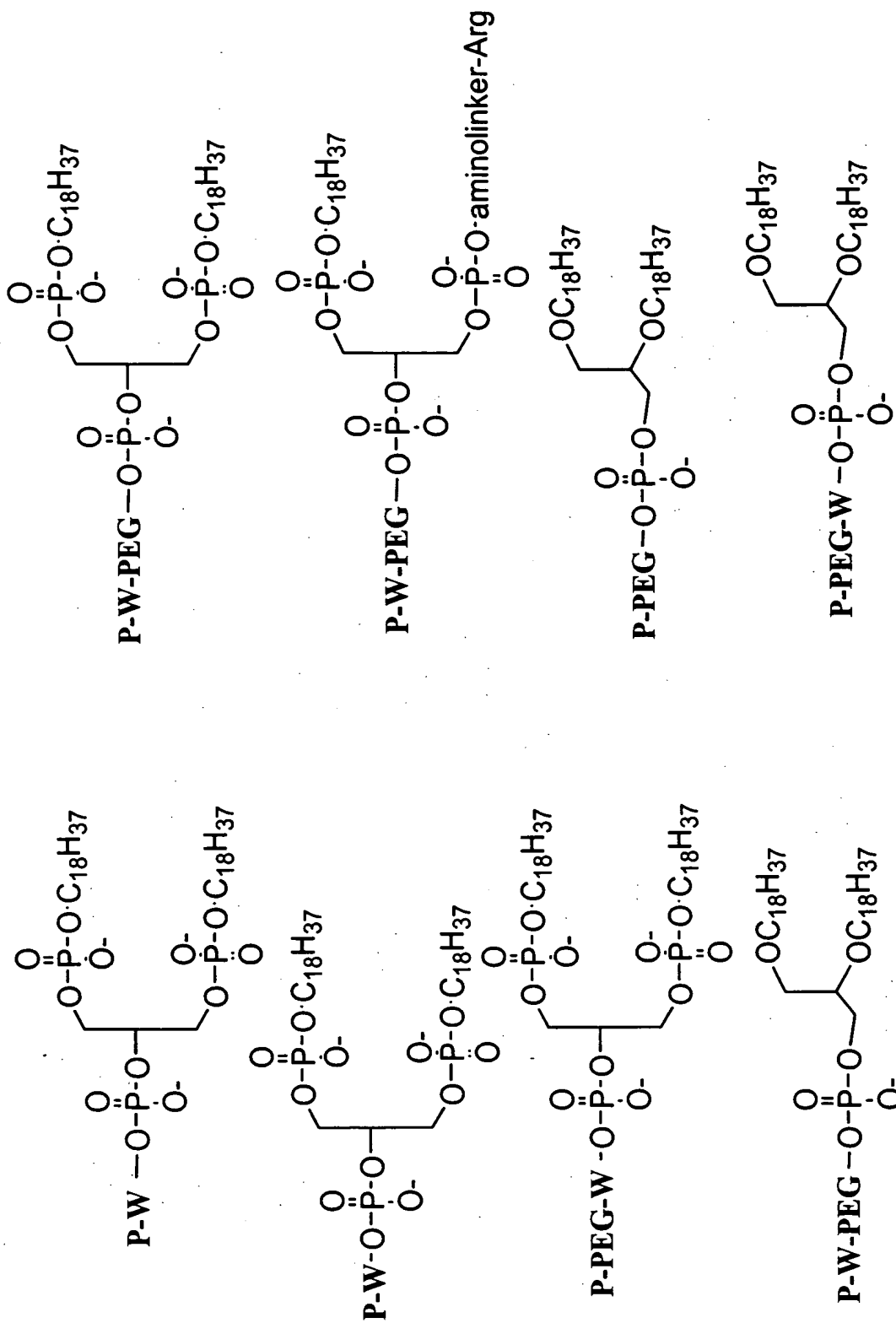
NMI = N-METHYLMIDAZOLE OR OTHER ACTIVATOR

Figure 26b: Protein PEG conjugate with cleavable linker



- $B_1 = \text{T, U}$
 $B_2 = \text{C, U, A}$
 $R_1 = \text{O, S}$
 $R_2 = \text{O, S, N, alkyl etc.}$
 $R_3 = \text{O, S, N, C}$
 $R_4 = \text{O, S, N, C}$
 $R_5 = \text{H, OH, O-alkyl etc.}$
 $R_6 = \text{substituted nitrogen, eg. N(iPr)}_2$
 $R_7 = \text{O or S alkyl, cyanoalkyl, eg. cyanoethyl}$

Figure 27: Peptide or Protein/Phospholipid Conjugates

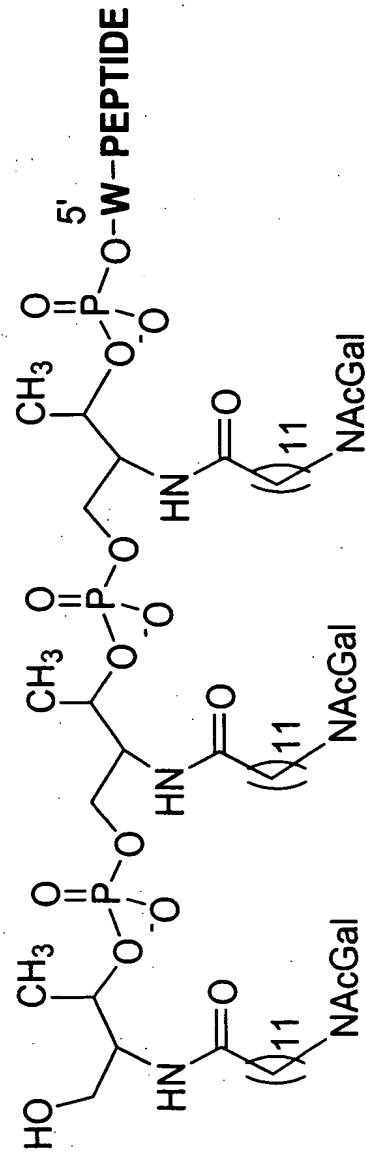


PEG=polyethylene glycol

W=cleavable linker (e.g. A-dT, C-dT)

P= Peptide/Protein

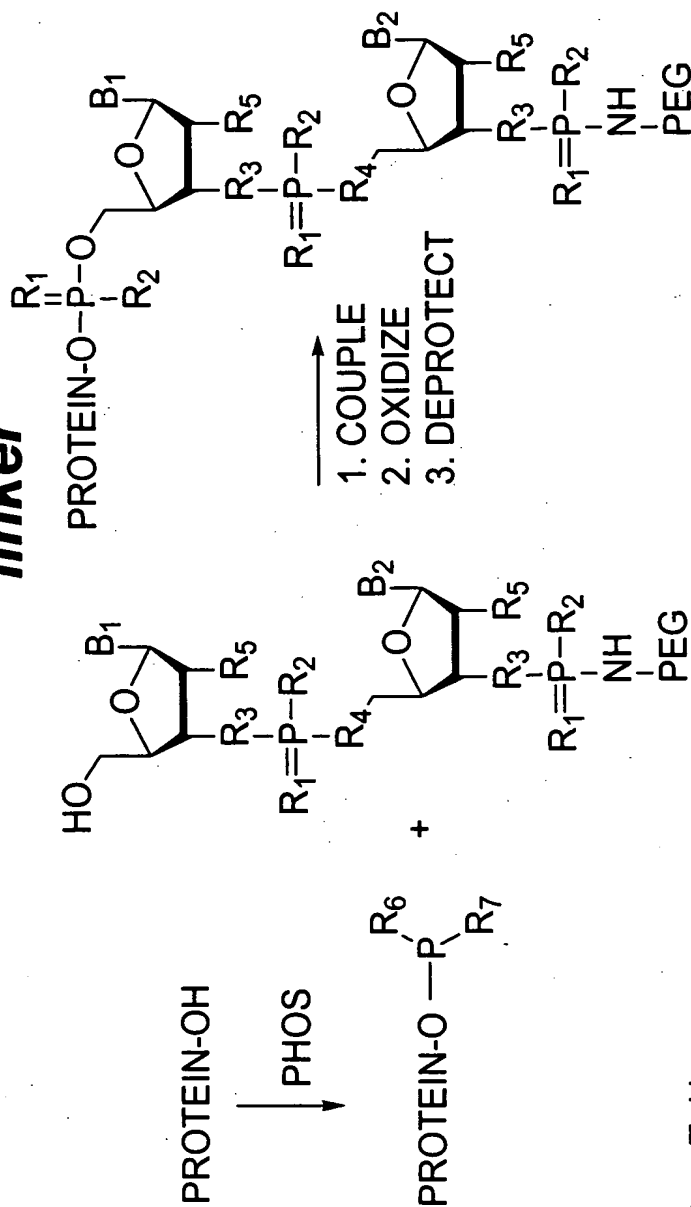
Figure 28: Conjugation of targeting ligands to a peptide or protein



N-acetyl-D-galactosamine conjugate

W = cleavable linker (eg. A-dT, C-dT dimer)

Figure 29: Protein/PEG conjugate with cleavable linker



B₁ = T, U
 B₂ = C, U, A
 R₁ = O, S
 R₂ = O, S, N, alkyl etc.
 R₃ = O, S, N, C
 R₄ = O, S, N, C
 R₅ = H, OH, O-alkyl etc.

R₆ = substituted nitrogen, eg. N(iPr)₂
 R₇ = O or S alkyl, cyanoalkyl, eg. cyanoethyl
 PHOS = PHOSPHITYLATION

Figure 30: siNA Cholesterol Conjugate

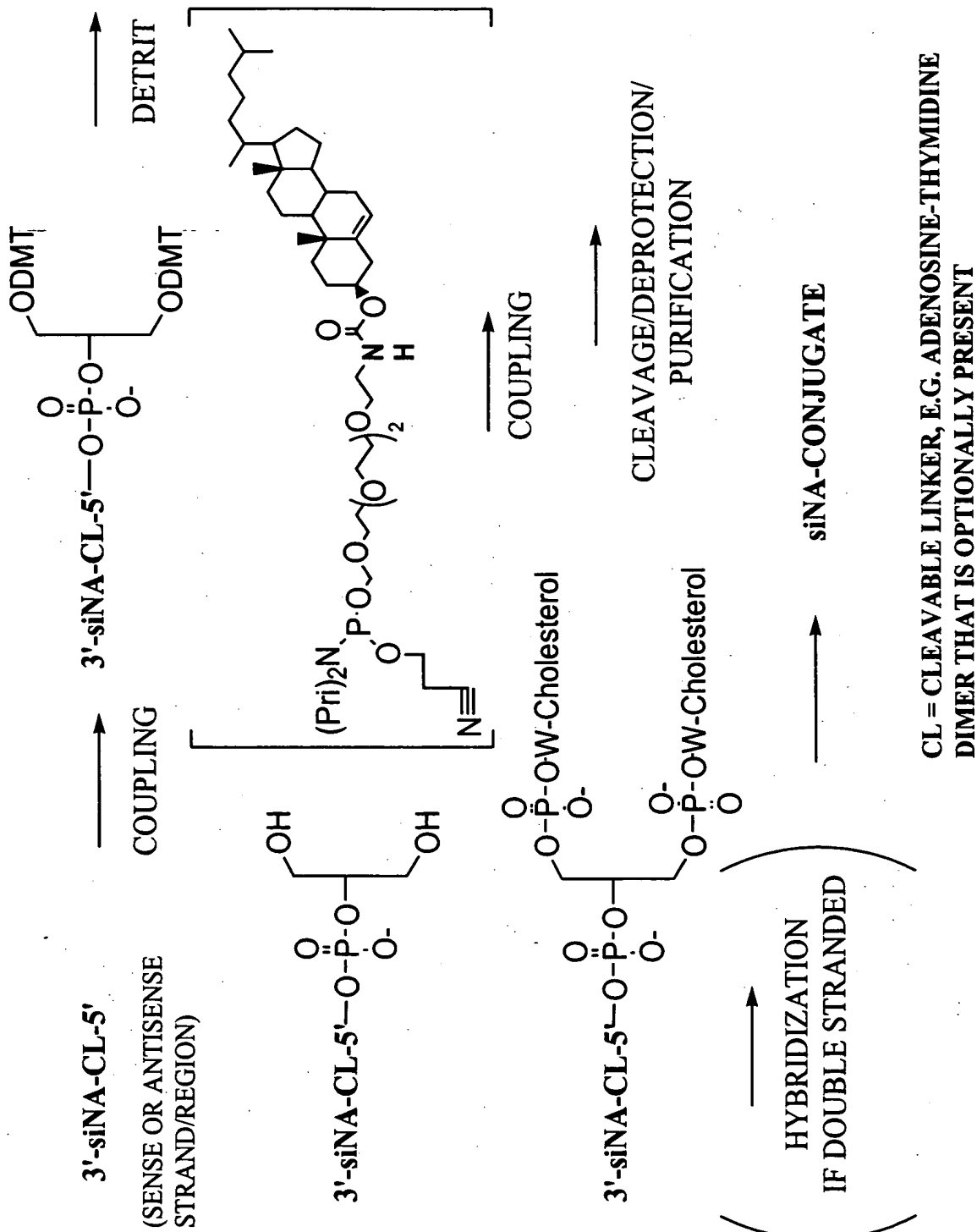
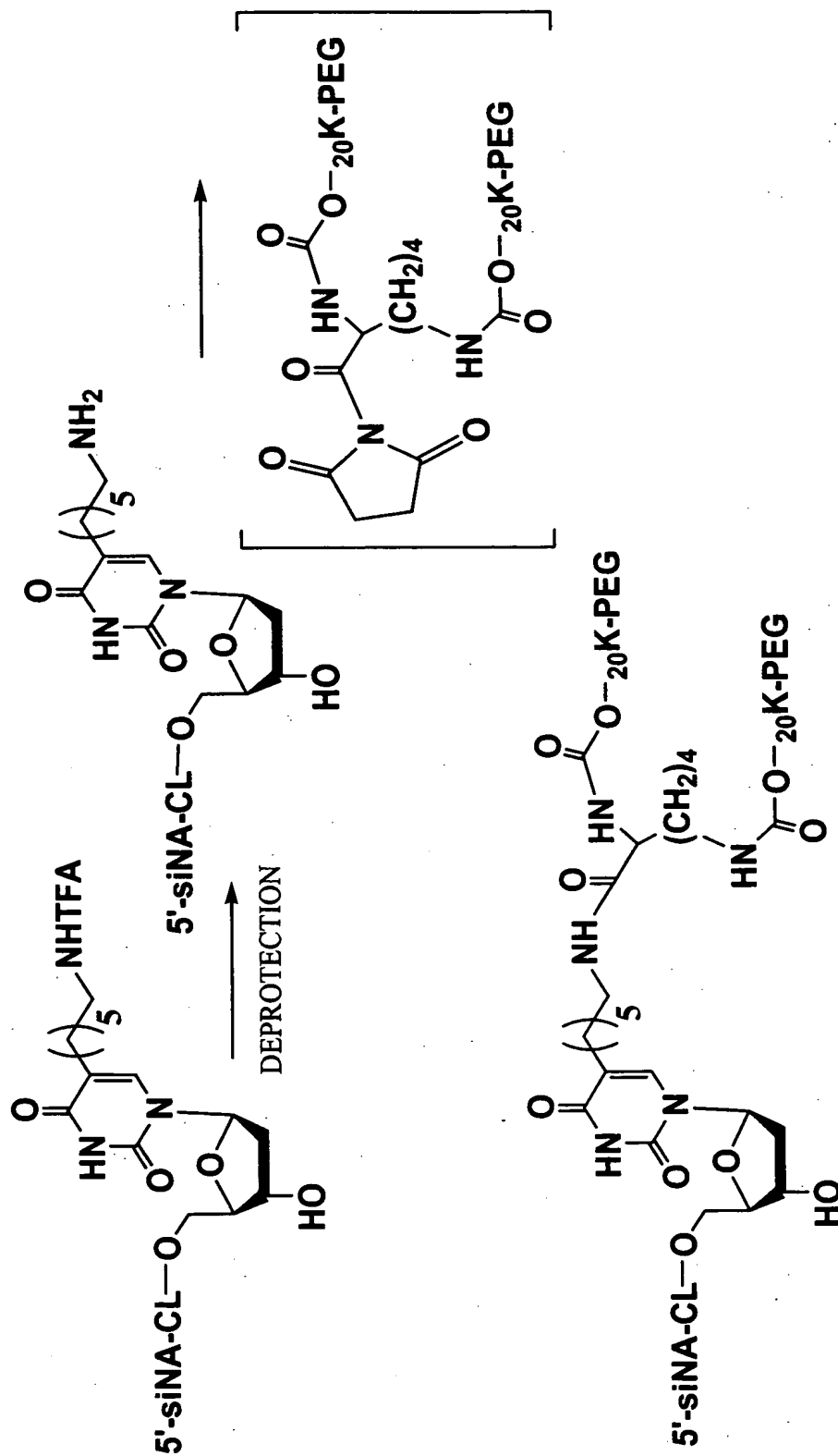


Figure 31: siNA 3'-PEG Conjugate



CL = CLEAVABLE LINKER, E.G. ADENOSINE-THYMIDINE DIMER
 THAT IS OPTIONALLY PRESENT

Figure 32: siNA 3'-Cholesterol Conjugate

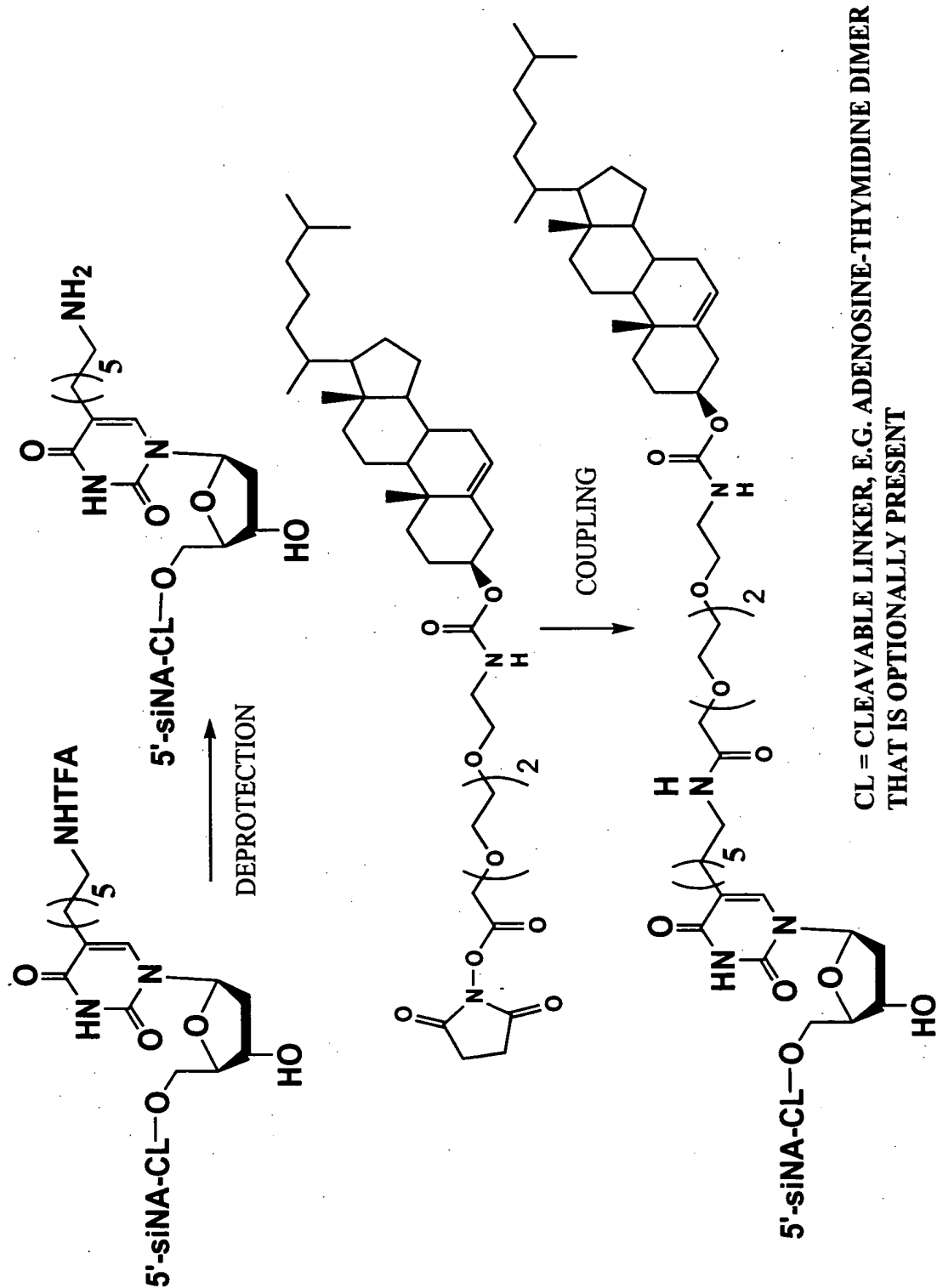
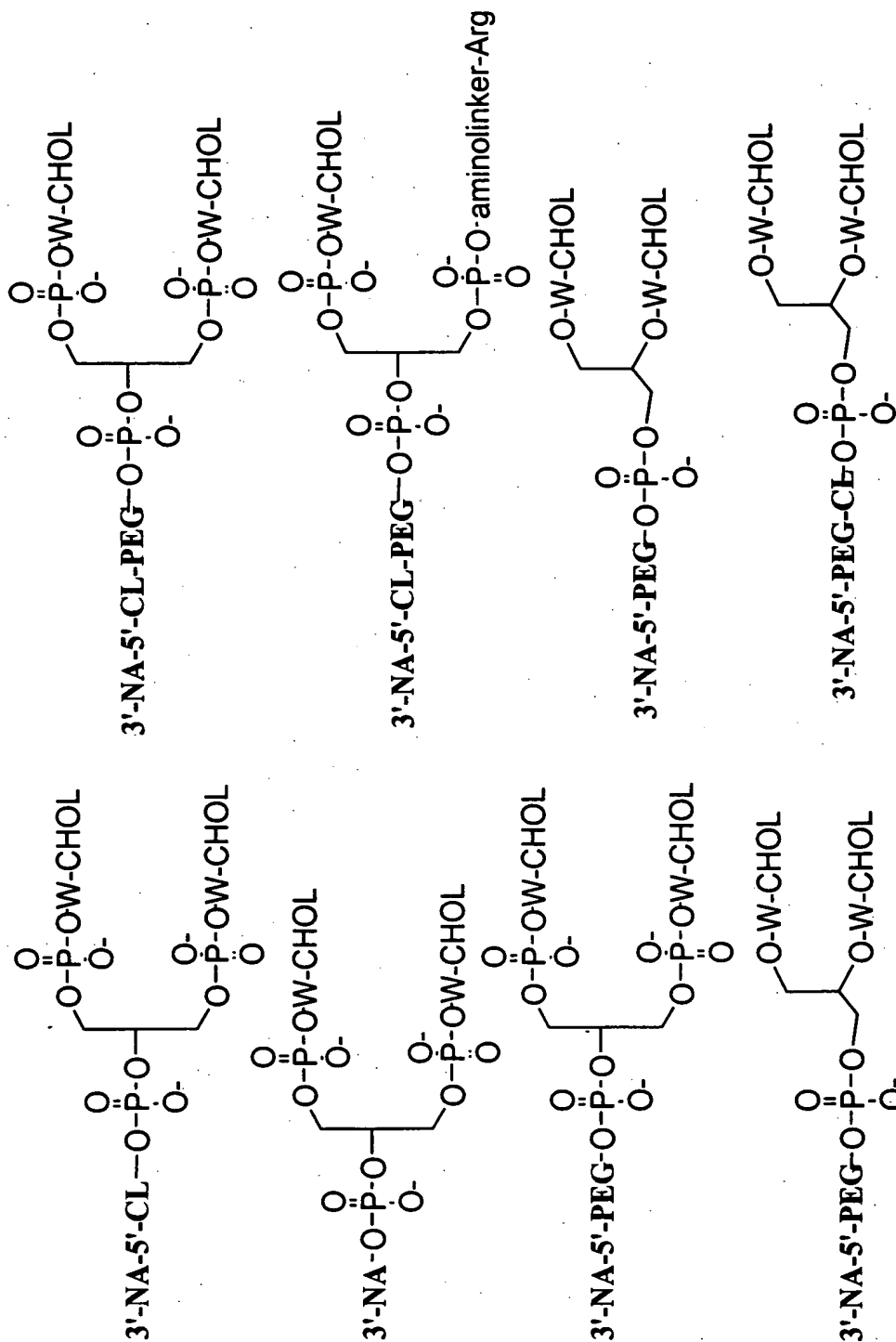


Figure 33: Nucleic Acid Cholesterol Conjugates



PEG=polyethylene glycol

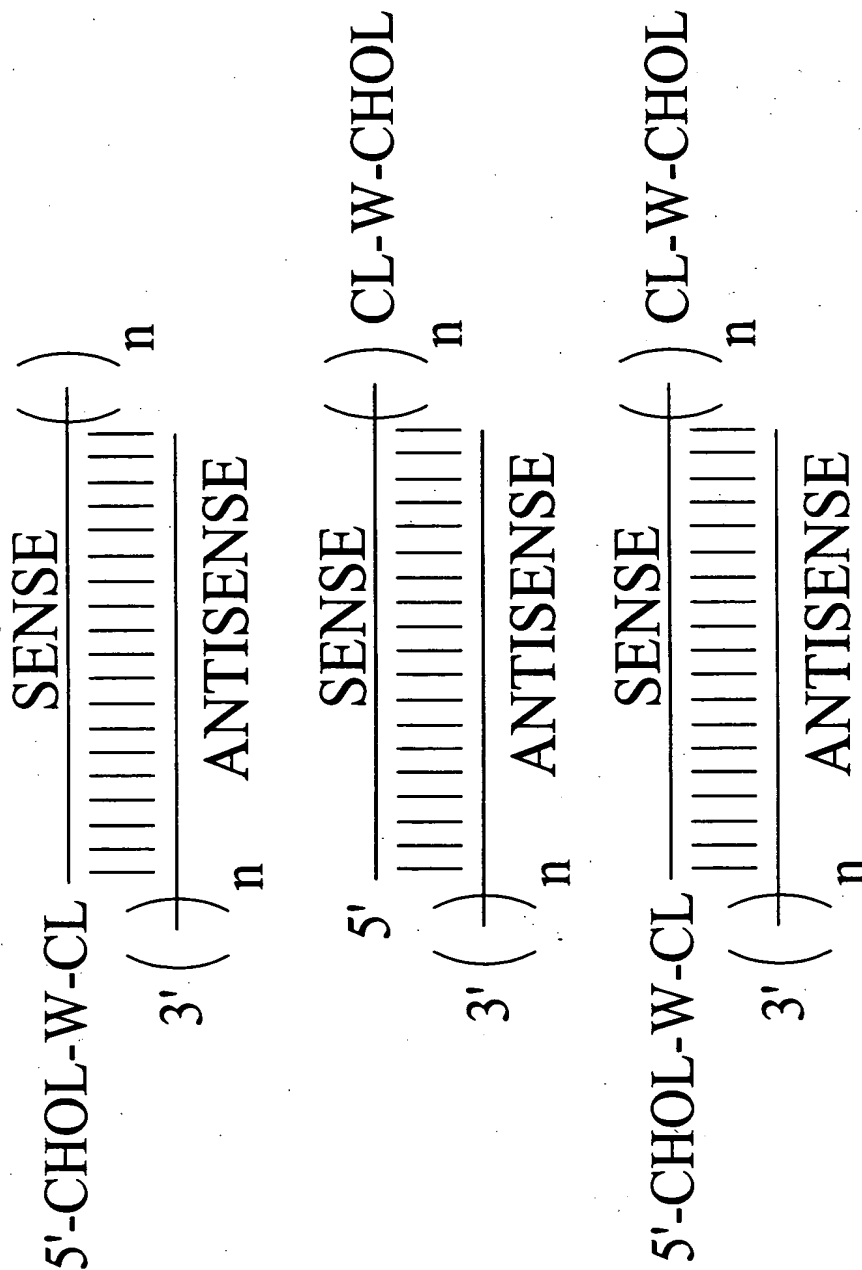
CL=cleavable linker (e.g. A-dT, C-dT)

NA= Nucleic Acid Molecule such as siNA, antisense, or enzymatic nucleic acid

CHOL=cholesterol or an analog or metabolite thereof

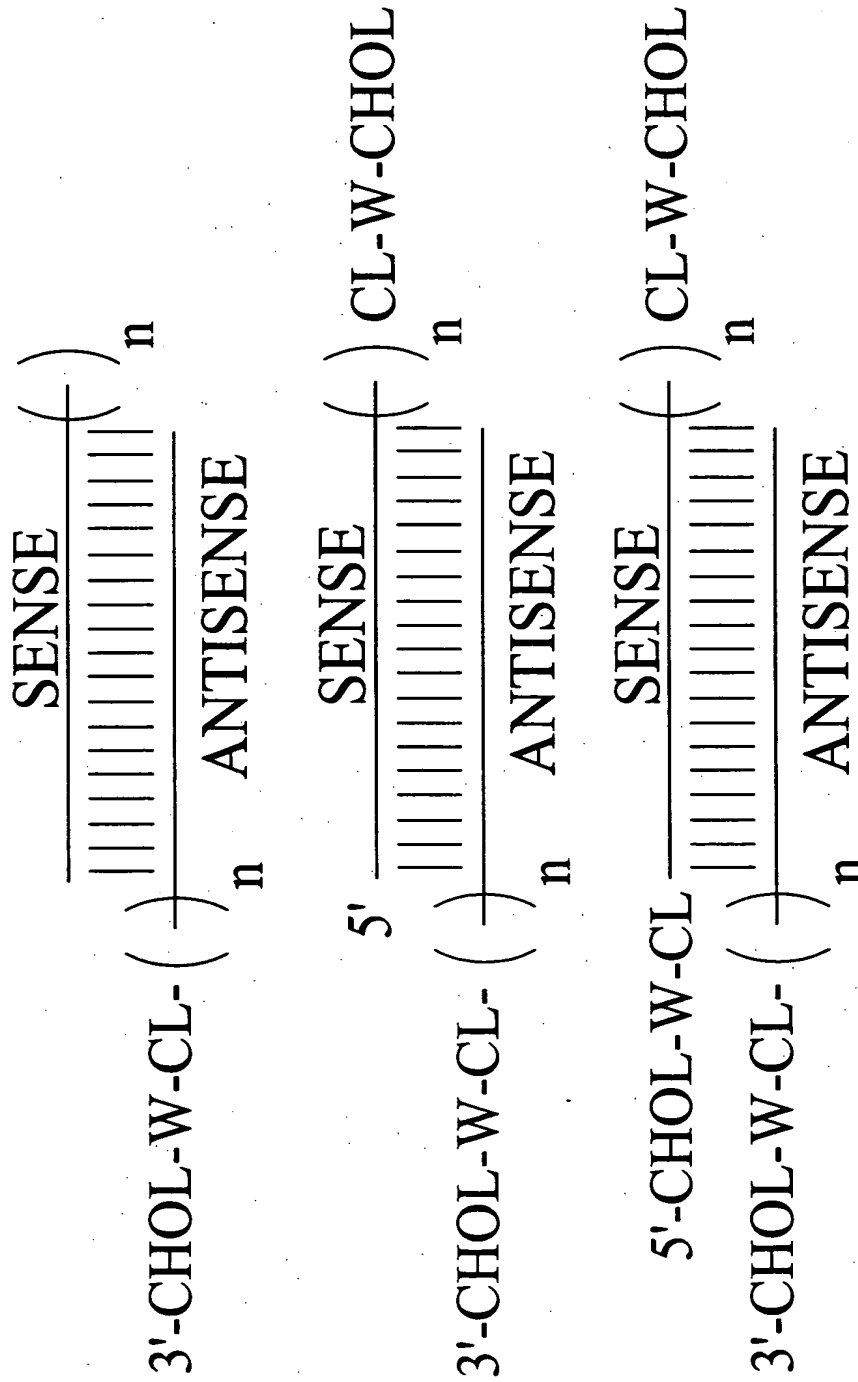
W= linker molecule (see for example Formulae 109 or 112)

Figure 34: siNA Cholesterol Conjugates



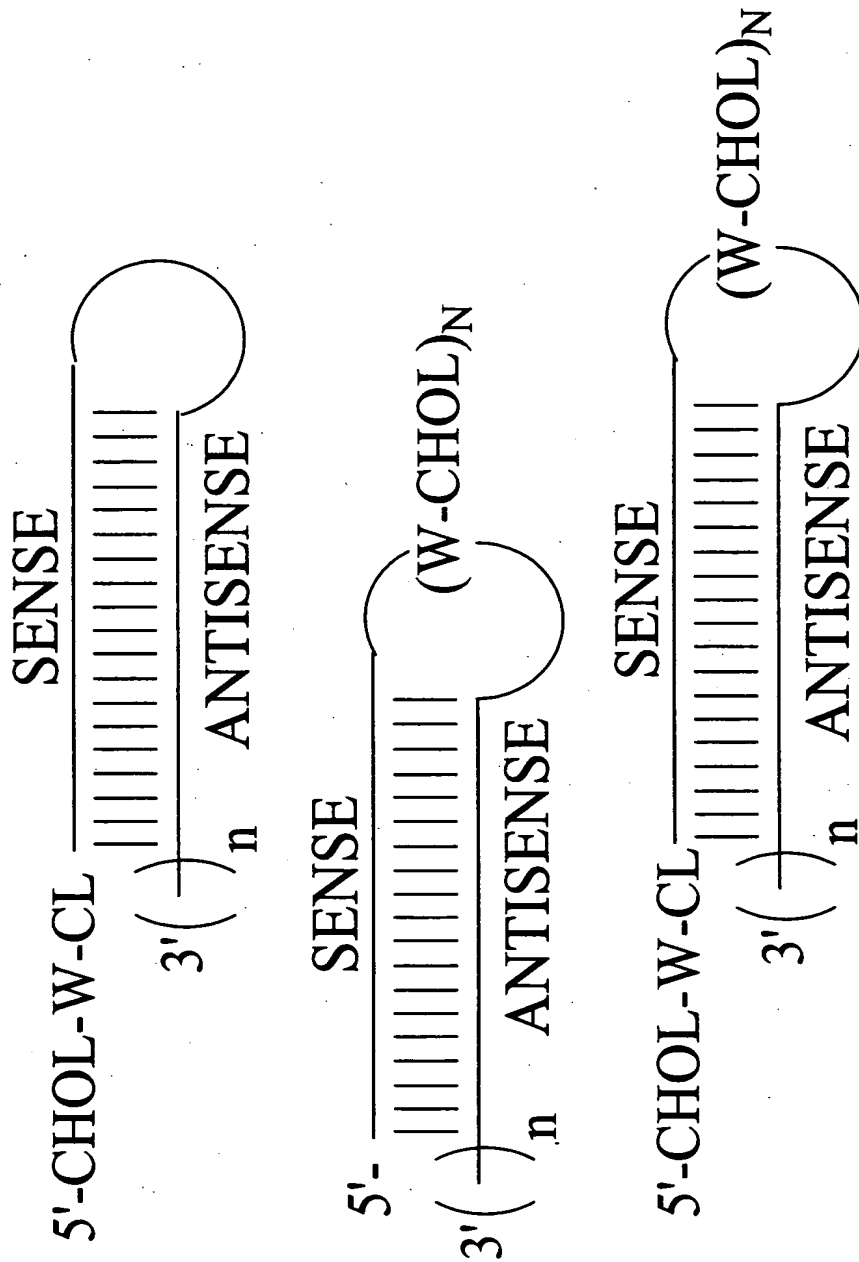
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
CHOL=cholesterol or an analog or metabolite thereof
W= linker molecule (see for example Formulae 107, 108, 109 or 115)
n = integer, e.g. 1, 2, or 3

Figure 35: siNA Cholesterol Conjugates



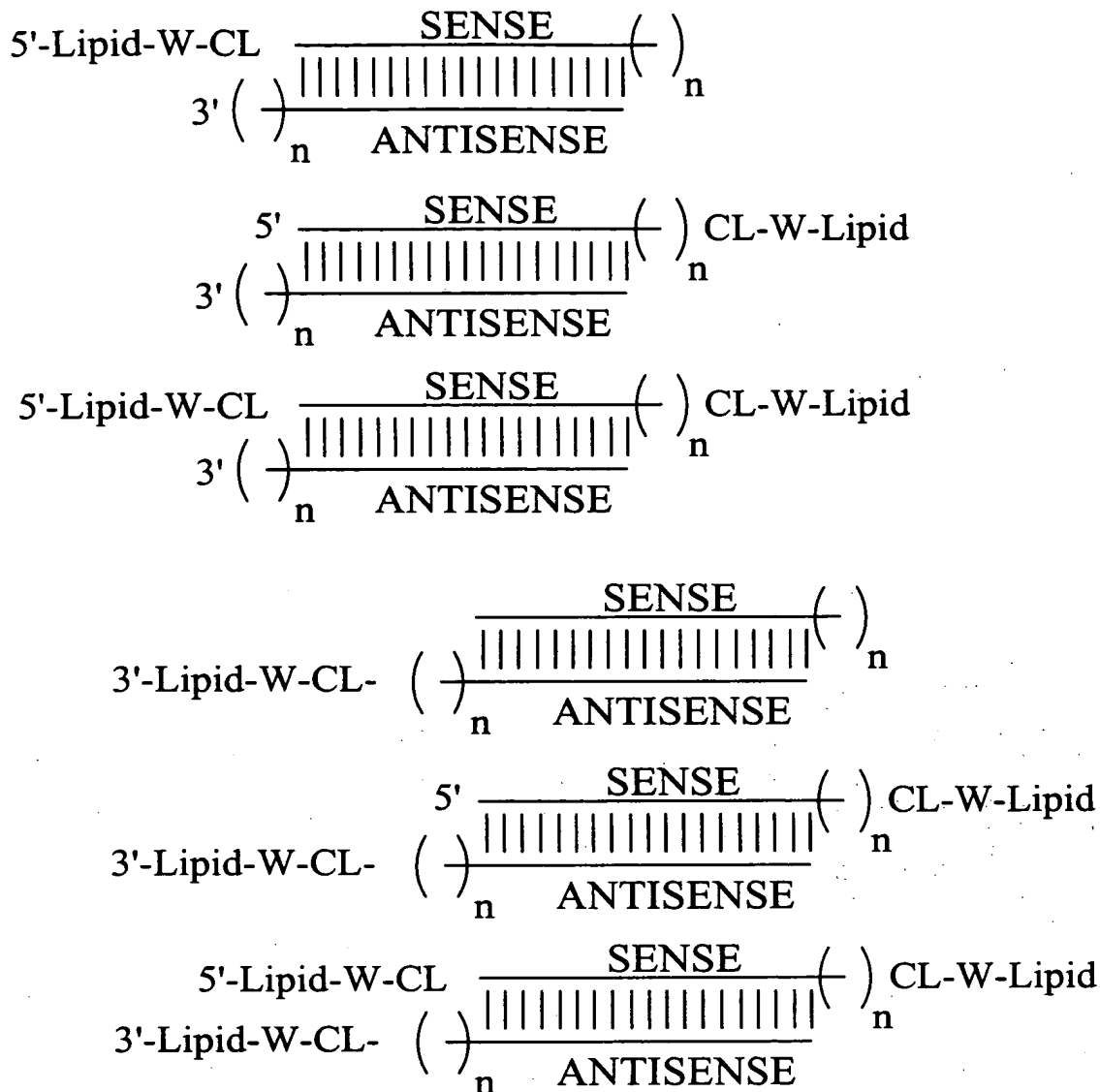
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
 CHOL=cholesterol or an analog or metabolite thereof
 W= linker molecule (see for example Formulae 107, 108, 109 or 115)
 n = integer, e.g. 1, 2, or 3

Figure 36: siNA Cholesterol Conjugates



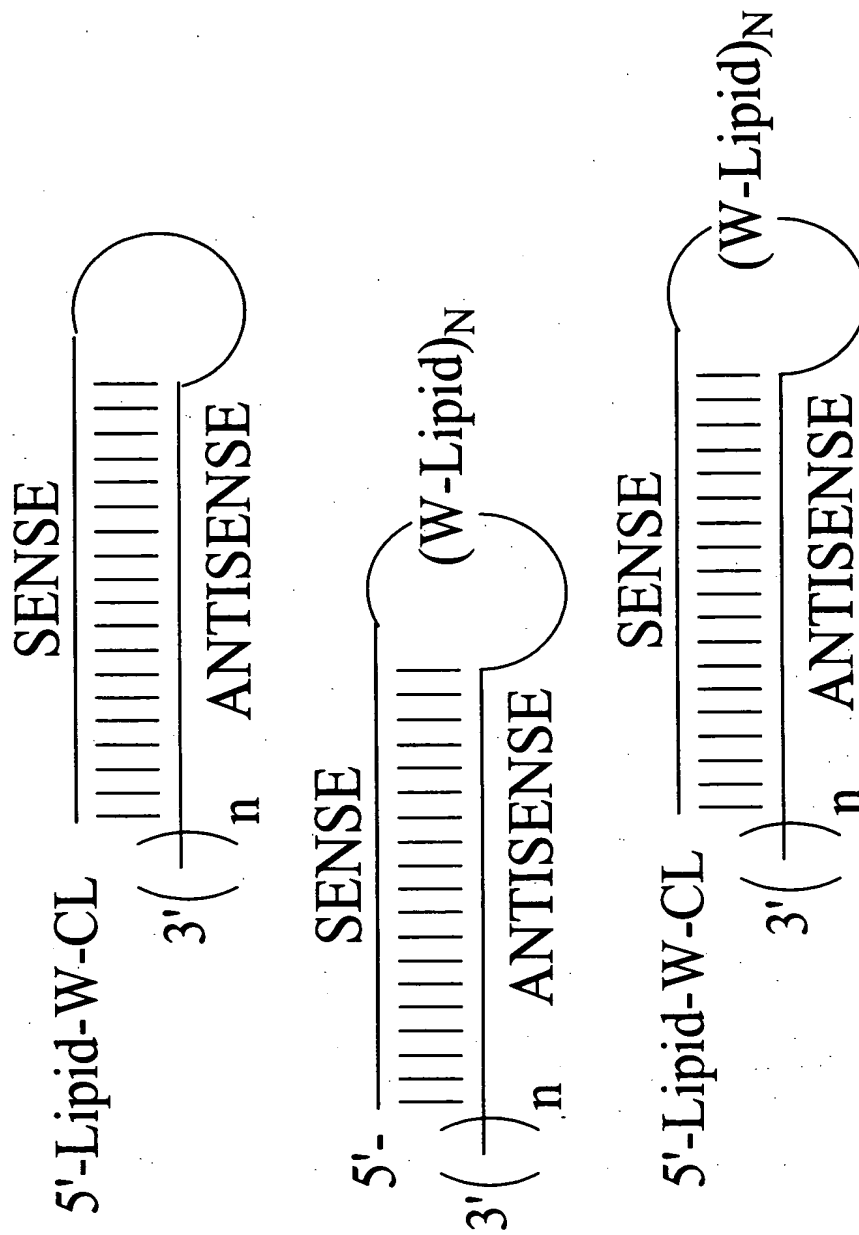
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
 CHOL=cholesterol or an analog or metabolite thereof
 W= linker molecule (see for example Formulae 107, 108, 109 or 112)
 n = integer, e.g. 1, 2, or 3
 N=integer, e.g. 1, 2, 3, or 4

Figure 37: siNA Lipid Conjugates



CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
Lipid=Straight chain or branched alkyl or fatty acid, e.g. C₁₈H₃₇
W= linker molecule (see for example Formulae 48, 49, 64, or 65)
n = integer, e.g. 1, 2, or 3

Figure 38: siNA Lipid Conjugates



CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present

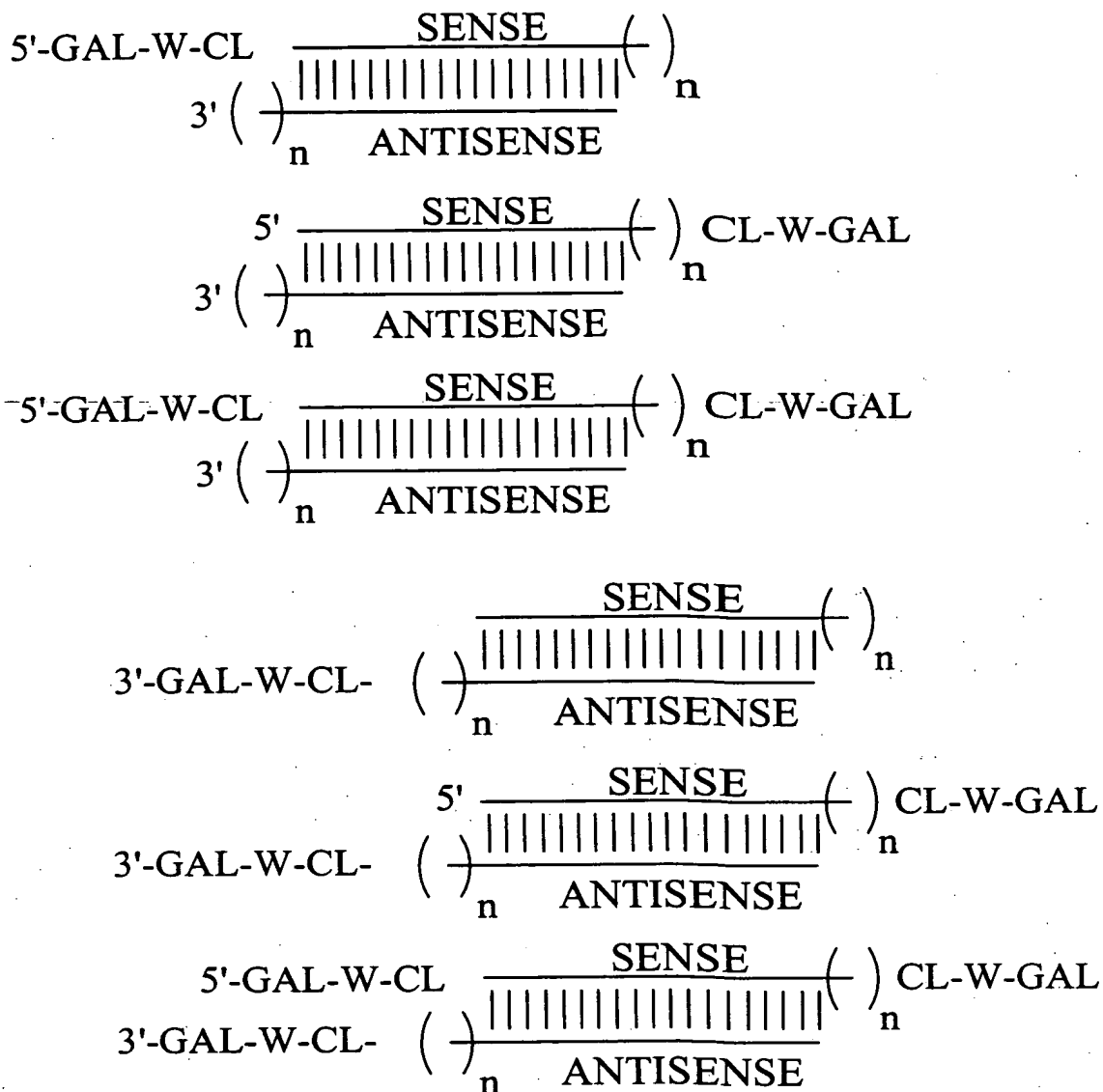
Lipid=Straight chain or branched alkyl or fatty acid, e.g. C₁₈H₃₇

W= linker molecule (see for example Formulae 48, 49, 64, or 65)

n = integer, e.g. 1, 2, or 3

N=integer, e.g. 1, 2, 3, or 4

Figure 39: siNA Galactosamine Conjugates



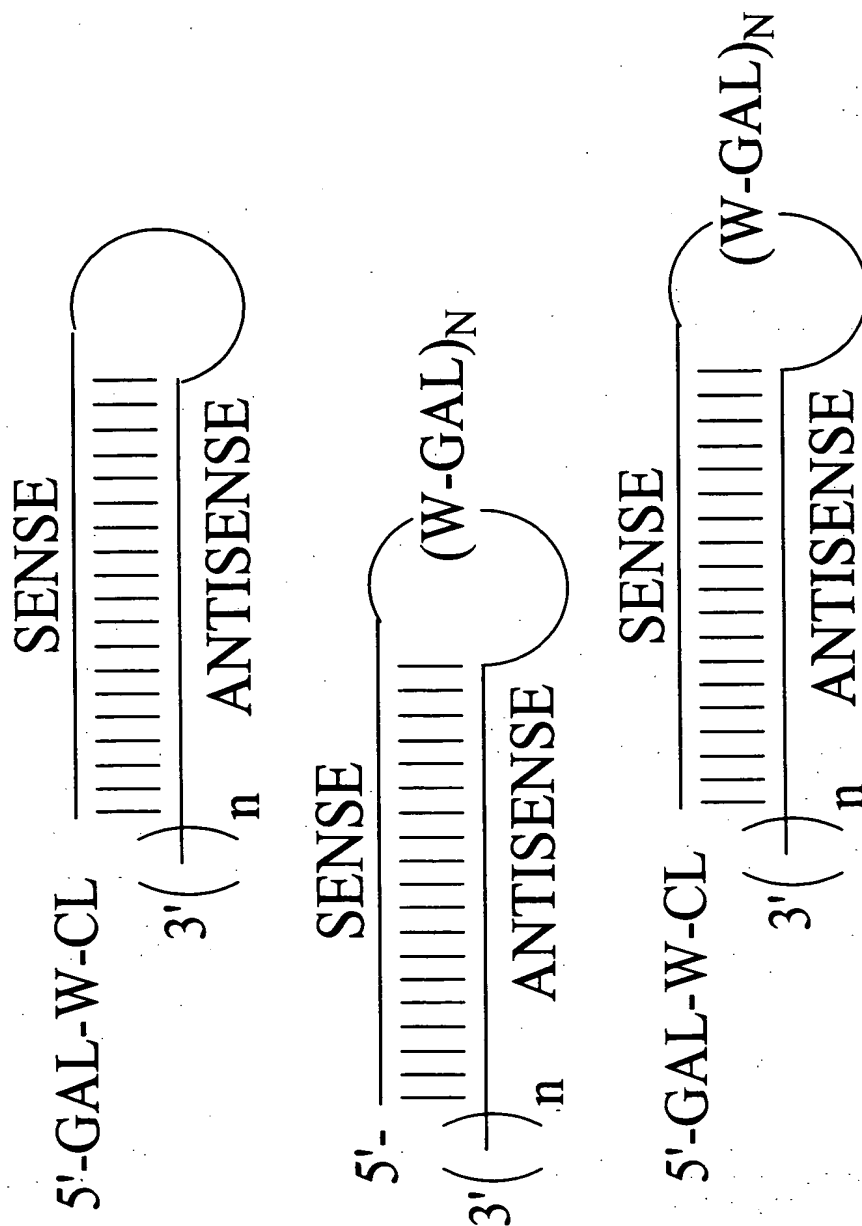
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present

GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106

W= linker molecule (see for example Formulae 102 or 103)

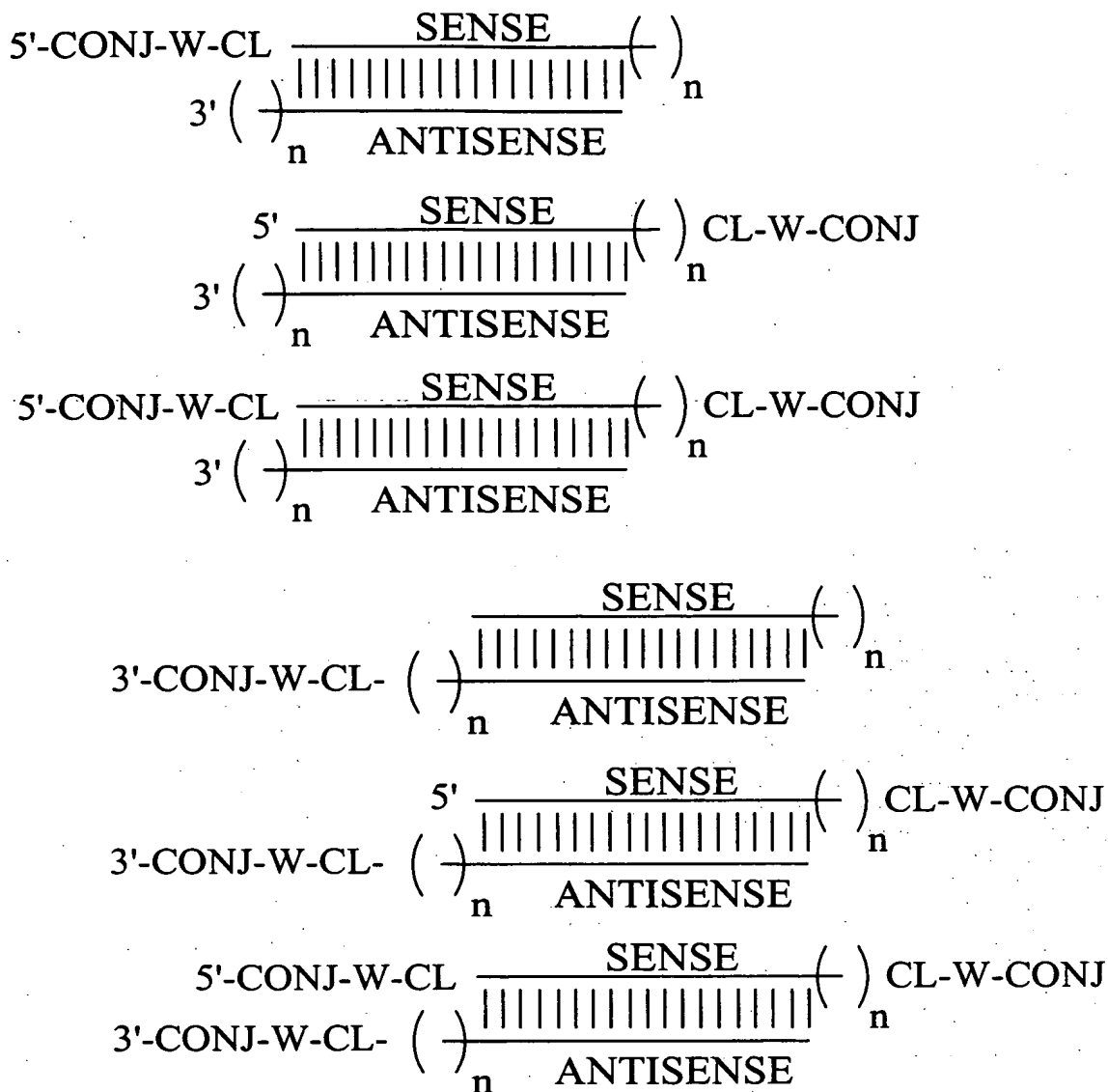
n = integer, e.g. 1, 2, or 3

Figure 40: siNA Galactosamine Conjugates



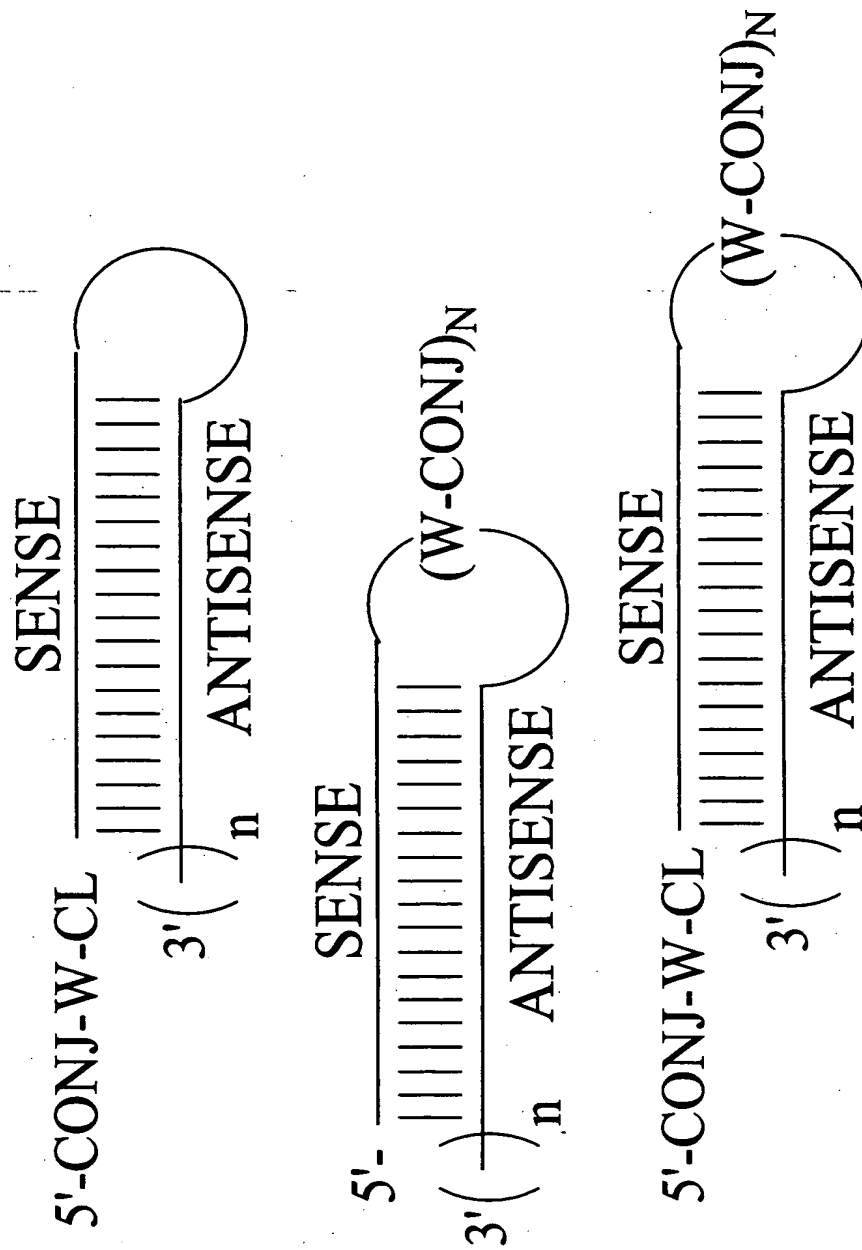
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
 GAL=GALACTOSAMINE; e.g. compounds having Formulae 51-56, 86, 92, 99, 100, 103, 105, 106
 W= linker molecule (see for example Formulae 102 or 103)
 n = integer, e.g. 1, 2, or 3
 N=integer, e.g. 1, 2, 3, or 4

Figure 41: Generalized siNA Conjugate Design



CONJ=any biologically active molecule or conjugate as described herein
CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present
W= linker molecule
n = integer, e.g. 1, 2, or 3

Figure 42: Generalized siNA Conjugate design



CONJ=any biologically active molecule or conjugate as described herein

CL=cleavable linker (e.g. A-dT, C-dT) that is optionally present

W= linker molecule

n = integer, e.g. 1, 2, or 3

N=integer, e.g. 1, 2, 3, or 4

Figure 43: Distribution of Intact siNA in Liver After SC Administration of Conjugated or Unconjugated Chemistries

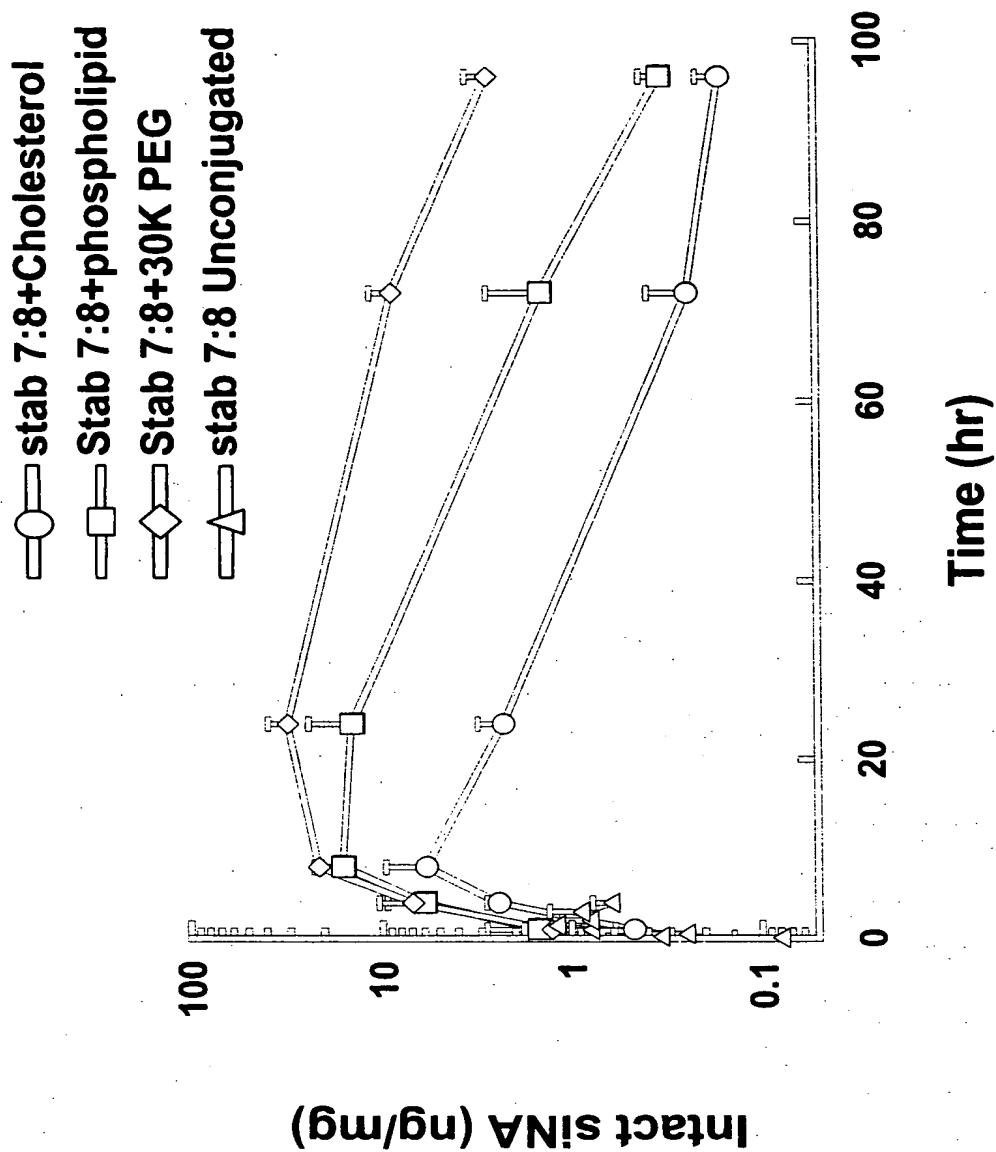
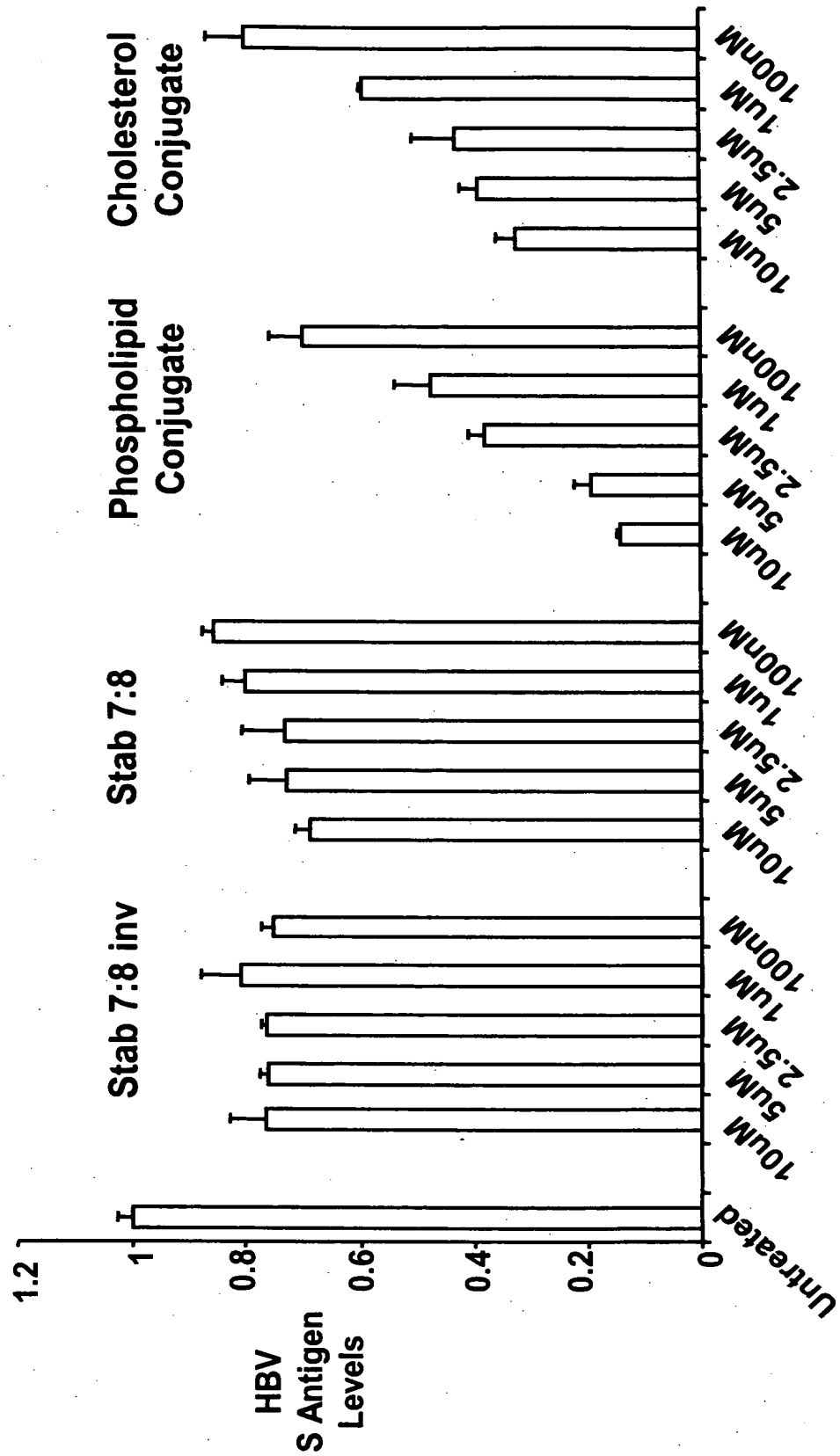


Figure 44: Lipid Free Delivery of HBV siNA Conjugates in Cell Culture





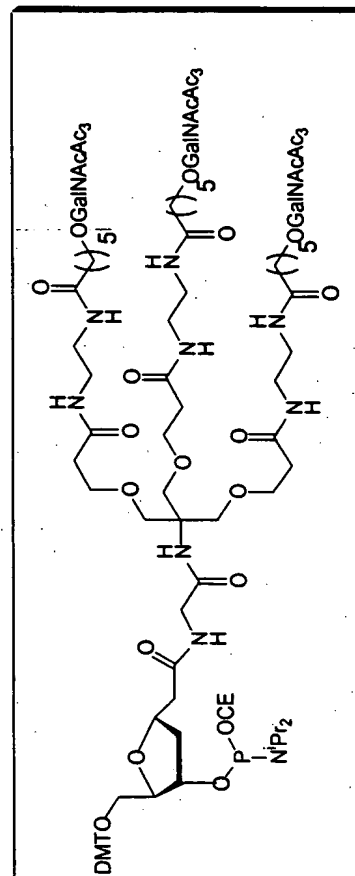
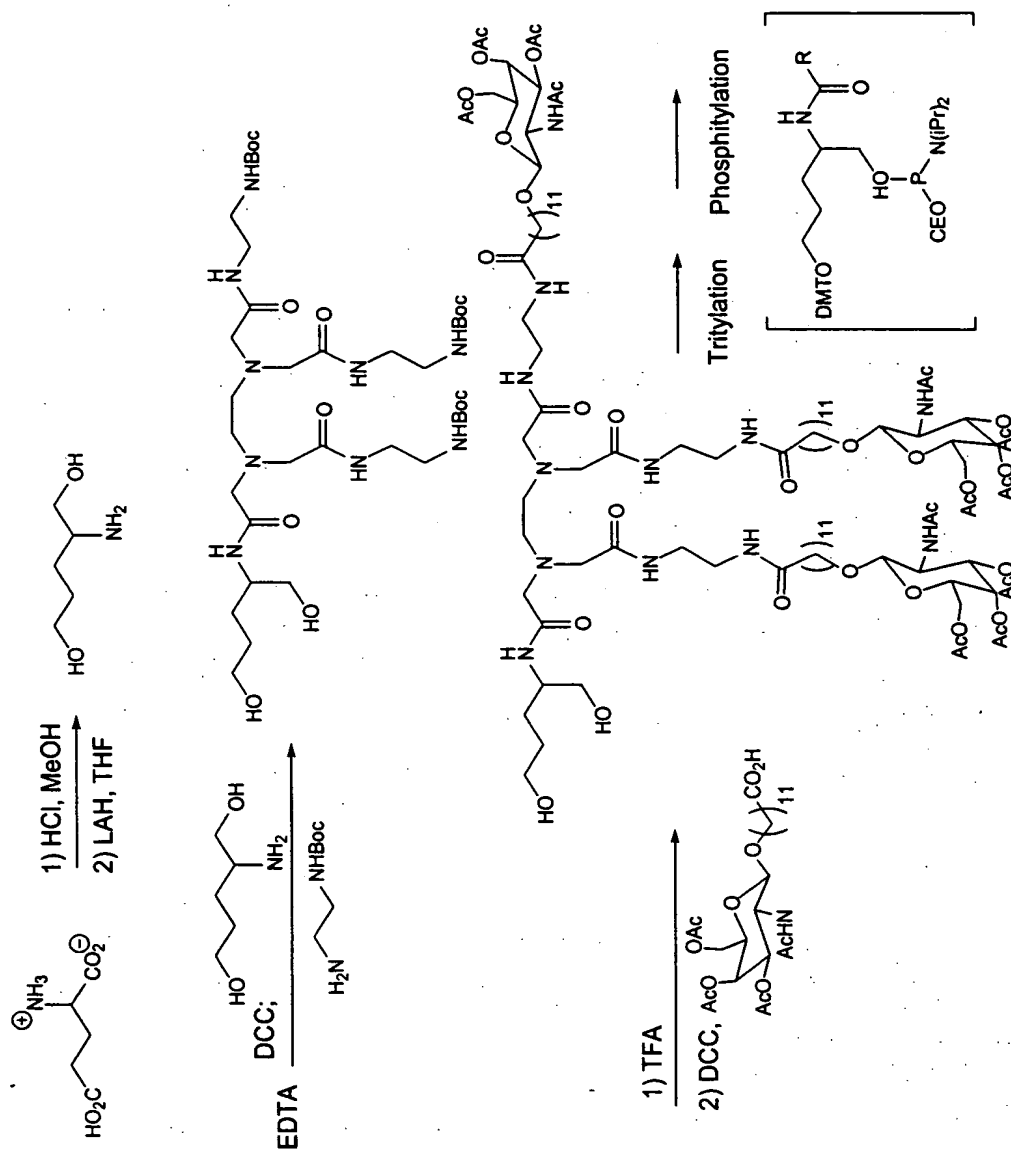


Figure 47: Synthesis of another Tri-Galactosamine Conjugate



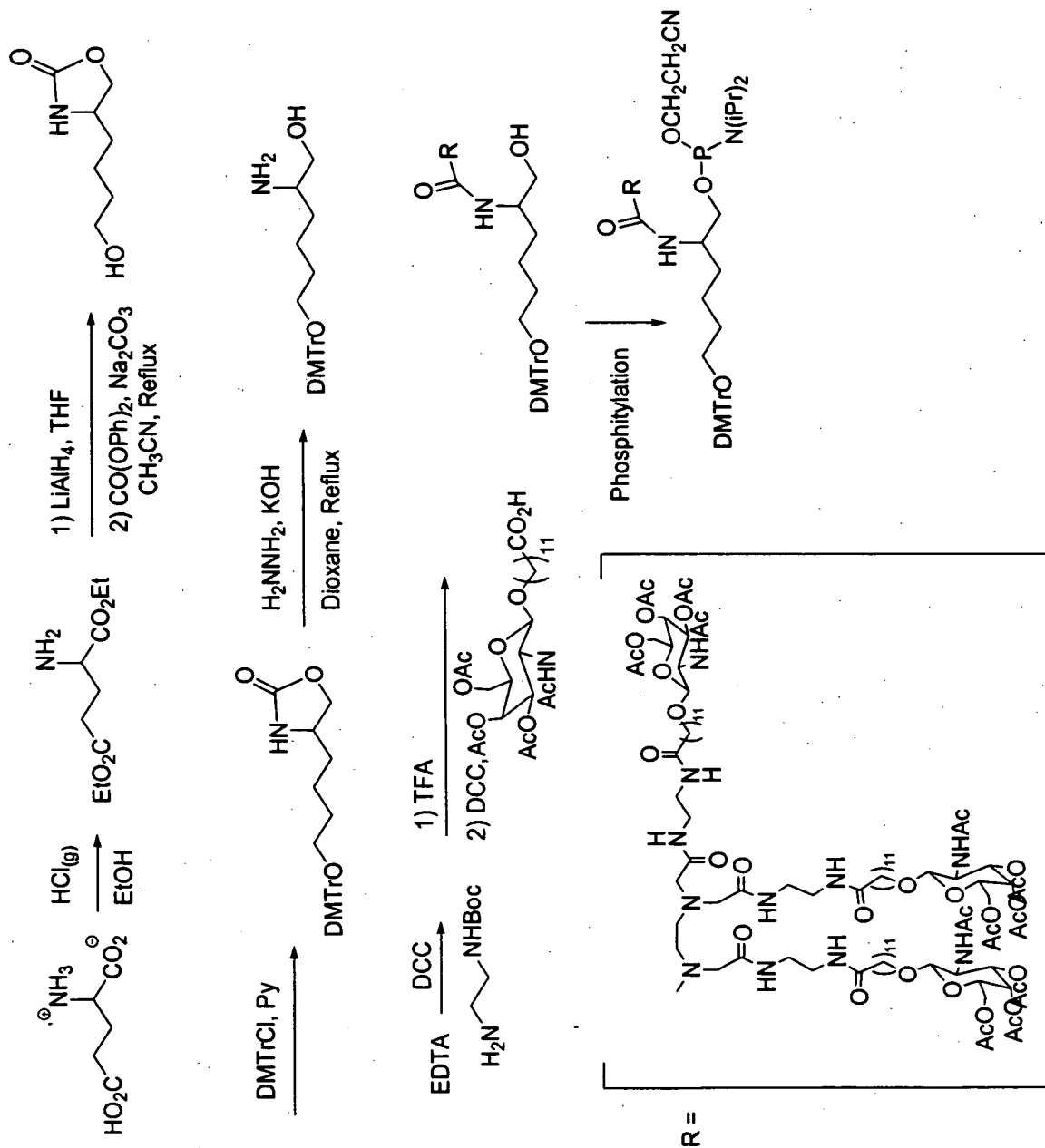


Figure 49: Synthesis of NHS Cholesterol Conjugate

